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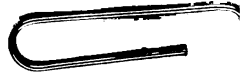
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TREASURY DEPARTMENT.
Public Health and Marine-Hospital Service of the United States.
WALTER WYMAN, Surgeon-General.

HYGIENIC LABORATORY.—BULLETIN No. 8.

AUGUST, 1902.

LABORATORY COURSE
IN
PATHOLOGY AND BACTERIOLOGY

BY
M. J. ROSENAU,
DIRECTOR OF HYGIENIC LABORATORY.



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LETTER OF TRANSMITTAL.

SIR : I have the honor to transmit herewith a syllabus of the course in pathology and bacteriology given in this laboratory.

This is the completion of the third year in which the course of instruction as outlined in the following pages has been given by the present director of the laboratory to the student-officers of the Service. Advantage has been taken of many practical points gained from actual experience both from the standpoint of the student and that of the instructor, so that the course as now outlined is believed to fairly cover the ground and to require few changes other than those due to advances in our knowledge upon the subjects treated.

The object of the course is to thoroughly equip officers of the Service in the technique of pathology and bacteriology so as to fit them better for the hospital, quarantine, epidemic, and public-health work of the Service. Officers of the Service who have taken the prescribed course are thoroughly prepared to make the scientific diagnoses of plague, cholera, diphtheria, tetanus, tuberculosis, typhoid fever, anthrax, and other communicable diseases, as well as to carry on original investigations for themselves.

The course as outlined requires about one year for its completion. The student-officer is required to work each subject until he has mastered it before going on to the next. Such deliberation permits a thoroughness which is not possible when work is hurried or limited to a short space of time.

The exact sequence as given on the following pages is not strictly adhered to, because it is the desire of the director to make the course as practical as circumstances will permit. Therefore, the study of diphtheria is usually left until the fall of the year or winter, when practical experience may be had with the cases which usually occur in the District. The studies upon vaccinia and smallpox are taken up in the winter, which time is more apt to offer material and cases. Malarial work is of course confined to the summer and fall seasons. In connection with the health department of the District, the work of diagnosing diphtheria from swabs and culture tubes and the administration of anti-toxin, both for prophylactic and curative purposes, is carried on by each student-officer. In connection with the studies upon vaccinia, a visit is always made to one of the large vaccine farms, which offers special advantages for observing the process of vaccinating the calves, obtaining the lymph and pulp, glycerinating the virus, and preparing

the product for the market. In like manner advantage is taken, as far as it is practicable, of the material in hospitals and other institutions situated in the city of Washington.

It will be noticed that the course apparently deals more with the subject of bacteriology than that of pathology. The latter, however, is not neglected. Owing to circumstances, it is not possible at present to treat pathologic-histology in the same systematic manner as bacteriology. The pathologic-histology of all the infectious processes studied in the laboratory are thoroughly worked. Tumors and the special pathology of the organs are taken up from time to time as the specimens are received. Experience has taught that this is better than to attempt to give a systematic course in general and special pathology, in which branches the student-officer usually comes to the laboratory with a good foundation.

It will also be noticed that considerable importance is given to the subject of disinfections and disinfectants. This arises from the fact that officers of the Service are required not only in their hospital work, but more especially in their quarantine and public health duties, to thoroughly understand the theory and practice of this important subject. Each student-officer is required to master the theory and mechanics of all the important disinfecting apparatus and to make actual tests upon the efficiency and practical value of each one.

The scientific workers in the laboratory meet one evening in each week in order to review the work of the week and to discuss the current literature. The journals that are received are assigned to each student-officer, and it is his duty to present a critical review of important articles which he reads. We have found this an admirable plan for keeping thoroughly abreast with the large amount of literature constantly appearing, as well as a stimulus to thought and work.

After the student-officer has been in the laboratory several months and has thoroughly acquainted himself with the necessary technique, opportunity is given him to assist with the work of the laboratory, and every encouragement is offered to work out original problems of his own.

The classes have been small in number, thus allowing for the individual equation of each student-officer, as well as a maximum of personal attention.

The bacteriologic nomenclature adopted in this bulletin is based upon Migula's recent *System der Bakterien*, 1900, and I am indebted to Dr. Ch. Wardell Stiles for valuable assistance in determining many points in this difficult and much-neglected subject.

Respectfully,

M. J. ROSENAU.

Passed Assistant Surgeon and Director Hygienic Laboratory.

THE SURGEON-GENERAL.

LABORATORY COURSE IN PATHOLOGY AND BACTERIOLOGY.

HYGIENIC LABORATORY,

United States Public Health and Marine-Hospital Service, Washington, D. C.

[By Milton J. ROSENAU, Passed Assistant Surgeon, United States Public Health and Marine-Hospital Service,
and Director of the Hygienic Laboratory.]

PREPARATION OF MEDIA—

1. Bouillon :
 - a. Acid.
 - b. Neutral.
 - c. Alkaline.
2. Sugar free bouillon (colon fermentation).
3. Glucose bouillon.
4. Lactose bouillon.
5. Dunham's solution.
6. Gelatin.
7. Agar-agar :
 - a. Glycerin agar.
 - b. Litmus agar.
8. Potato.
9. Blood-serum :
 - a. Fluid.
 - b. Coagulated.
 - c. Löffler's blood-serum mixture.
10. Milk.
11. Litmus milk.
12. Aqueous humor of ox eyes.
13. Ascitic fluid.
14. Urine.

STUDIES FOR MICROSCOPIC PRACTICE—

1. Diatoms.
2. Algae.
3. Protozoa.
4. Starch bodies.
5. Vegetable cells.
6. Cotton fibers.
7. Linen fibers.
8. Silk fibers.
9. Feathers.
10. Hair of man and of lower animals.
11. Scales of butterflies and of other insects.
12. Milk.
13. Air bubbles.
14. Oil drops.
15. Vinegar eels (*Anguillula aceti*).
16. Tube casts.
17. Ciliated epithelium.
18. Crystals of sugar, salt, uric acid, cholesterin, hematin, and various chemicals.
19. Decomposing meat.
20. Hay infusion.
21. Surface waters.

SCOPY—

STUDY OF SAPROPHYTES.

BACTERIA.

Genus *Bacillus* Cohn—

1. *Bacillus subtilis* (Ehrenberg, 1833) Cohn, 1872.
2. *Bacillus vulgaris* (Hauser, 1885) Migula, 1900. [*Proteus vulgaris*.]
3. *Bacillus megaterium* de Barry, 1884.
4. *Bacillus prodigiosus* (Ehrenberg, 1839) Flügge, 1886.

Genus *Pseudomonas* Migula—

5. *Pseudomonas fluorescens* Migula, 1900. [*Bacillus fluorescens liquefaciens* Flügge, 1886.]
6. *Pseudomonas aeruginosa* (Schröter, 1872) Migula, 1900. [*Bacterium aeruginosum* Schröter, 1872; *Bacillus pyocyaneus* Gessard, 1882.]

Genus *Planosarcina* Migula—

7. *Planosarcina agilis* (Ali-Cohen, 1889) Migula, 1900. [*Micrococcus agilis* Ali-Cohen, 1889.]

Genus *Sarcina* Goodsir—

8. *Sarcina lutea* Schröter, 1886.

Genus *Microspira* Schröter, 1886—

9. *Microspira aquatilis* (Günther, 1892) Migula, 1900. [*Vibrio aquatilis* Günther 1892.]

COMMON MOLDS—

Oidium.
Aspergillus.
Penicillium.
Mucor.

YEASTS—

Beer yeast.
 Red yeast.
 Black yeast.

CLASSIFICATION OF SCHIZOMYCETES—

1. According to some characteristic feature :

- a. Chromogenic.
- b. Photogenic.
- c. Zymogenic.
- d. Saprogenic.
- e. Pyogenic.
- f. Thiogenic.

2. According to morphology :

Cocci—

- a. Micrococci.
- b. Diplococci
- c. Streptococci.
- d. Staphylococci.
- e. Tetrads.
- f. Sarcinae.
- g. Merismopedia.
- h. Ascococcus.

Bacilli—

- a. Leptothrix.
- b. Cladothrix.
- c. Streptothrix.

Spirilla—

- a. Vibrio.
- b. Spirillum.
- c. Spirochæta.

3. According to motion :

- a. Nonmotile.
- b. Motile.
- c. Brownian motion.

4. According to arrangement of flagella :

- a. Monotrichic.
- b. Amphitrichic.
- c. Lophotrichic.
- d. Peritrichic.

5. According to relation to spores :

1. Nonspore bearing.
2. Spore bearing—
 - a. Endospores.
 - b. Arthrospores.

MAKING PLATES.

1. Koch's method—glass plates.
2. Esmarch's roll tubes.
3. Slants of agar and gelatin in test tubes.
4. Petri dishes—
 Streaking on surface with platinum wire.
 Daubing with swabs on surface.
 Mixing while fluid.

OBTAINING PURE CULTURES—

1. By plate method on solid media.
2. By dilution in liquid media.
3. By temperature.
4. Inoculation into animals.
5. Negative plates.
6. Special methods.

OBSERVE GROWTH AND MULTIPLICATION OF BACTERIA IN CELL SLIDE—AGAR METHOD :

1. Division of bacilli.
2. Division of cocci.
3. Sporulation.

STUDY OF BROWNIAN MOVEMENT WITH :

India ink and other substances in hanging drop.

THE PRINCIPLES, CHEMISTRY, AND PRACTICE OF STAINING—

A. Vegetable stains :

1. Carmine—

- a.* Acid.
- b.* Alcoholic.
- c.* Alum.
- d.* Borax.

2. Hematoxylin—

- a.* Delafield's hematoxylin.
- b.* Alum hematoxylin.
- c.* Hemalum (Meyer).
- d.* Hematoxylin and eosin.

B. Aniline stains :

1. Acid stains—

- a.* Eosin.
- b.* Acid fuchsin.
- c.* Orange G.
- d.* Soudan.
- e.* Picric acid.

2. Basic stains—

- a.* Methylene blue (Löffler's solution).
- b.* Thionin.
- c.* Fuchsin (Carbol-fuchsin).
- d.* Gentian violet (Ehrlich's aniline water.)
- e.* Methyl violet.
- f.* Bleu d' argent.

C. Gram's method and solution :

D. Decolorization and differentiation :

- a.* Water.
- b.* Alcohol.
- c.* Aniline oil.
- d.* Hydrochlorate of aniline, 2 per cent.
- e.* Acids—organic and mineral.

E. Staining of spores :

Möller's method.

F. Staining of flagella :

- a.* Löffler's method.
- b.* Victoria blue.
- c.* Von Ermengem's method.

PRINCIPLES, CARE, AND USE OF INSTRUMENTS AND APPLIANCES—

- a. Camouflage.
- b. Camouflage.
- c. Photographic apparatus.
- d. Camouflage glass mask.
- e. Microscope.
- f. Paraffin bath.
- g. Analytical balance.
- h. Microscope.
- i. Thermometer.
- j. Thermoregulation: electric, neonatal, neonatal.
- k. Vacuum pumps.
- l. Blow pipe.
- m. Camera lucida.
- n. Spectroscope.
- o. X-ray tubes and induction coil.
- p. Autoclaves.
- q. Dry wall sterilizer.
- r. Arnold steam sterilizer.
- s. Brown serum sterilizer.
- t. Cold chamber.
- u. Sterilization of instruments.
- v. Sharpening and care of cutting instruments.

OPTIC PRINCIPLES INVOLVED IN MICROSCOPY—

1. Lenses:
 - a. Plano-convex.
 - b. Biconvex.
 - c. Concave.
 - d. Biconcave.
 - e. Doublets.
 - f. Triplets.
 - g. Quadruplets.
2. Objectives:
 - a. Achromatics.
 - b. Apochromatics.
 - c. Panchromatics.
3. Oculars:
 - a. Huyghenian.
 - b. Compensation.
 - c. Spectroscopic.
 - d. Micrometer:
 - e. Projection.
4. Condensers :
 - a. Simple—
 1. Biconvex.
 2. Plano-convex.
 - b. Abbe—

Numerical aperature 1.20.

Numerical aperature 1.40.
5. Microphotography and projection :
6. Micrometers :
 - a. Stage.
 - b Ocular.
 - c. Projection.

MEASURING MICROSCOPIC OBJECTS—

1. Eye-piece micrometer.
2. Stage micrometer.
3. Photographic method.

GLASS BLOWING—

1. The blowpipe.
2. Pipettes.
3. Bulbs.
4. Welding.
5. Sealing and opening glass containers.
6. Making test tubes.
7. Making perforated tubes for collodium sacks.

FOLDING PAPER FILTERS.**PREPARING BACTERIOLOGIC FILTERS (PRINCIPLES AND PRACTICE)—**

1. Kaolin.
2. Diatomaceous earth.
3. Asbestos.
4. Sand filtration.

PREPARATION AND MOUNTING OF HISTOLOGIC SPECIMENS—

1. Fixation :
 - a.* Acetic-bichlorid of mercury.
 - b.* Müllers fluid.
 - c.* Chrom-acetic acid.
 - d.* Flemming's solution.
 - e.* Formaldehyd.
 - f.* Absolute alcohol.
2. Dehydration :
 - a.* Alcohol 70 per cent (iodine alcohol if Hg Cl_2).
 - b.* Alcohol 85 per cent.
 - c.* Alcohol 95 per cent.
 - d.* Absolute alcohol, twice.
3. Clearing :
 - a.* Xylol.
 - b.* Chloroform.
 - c.* Turpentine.
 - d.* Oil cloves.
 - e.* Oil bergamot.
 - f.* Oil organum.
4. Infiltration and imbedding :
 - a.* Paraffin.
 - b.* Celloidin.
5. Sectioning :
 - a.* Single sections.
 - b.* Serial sections.
6. Attaching section to slide :
 - a.* Alcohol 85 per cent.
 - b.* Water.
 - c.* Myer's albumen.
 - d.* Gum Arabic.
 - e.* Collodium and clove oil.
7. Removal of imbedding material :
 - a.* Xylol to remove paraffin.
 - b.* Alcohol and water.
8. Staining :
 - a.* Nuclear staining.
 - b.* Protoplasmic.
 - c.* Regressive.
 - d.* Gram's.
 - e.* Bacterial.
 - f.* Special.
9. Dehydration in absolute alcohol, aniline oil :
10. Clearing :
 - a.* Xylol.
 - b.* Turpentine.
 - c.* Oil organum.
 - d.* Oil bergamot.
 - e.* Oil cloves.
 - f.* Chloroform.
11. Mounting in xylol balsam :

THE PARAFFIN BATH AND PARAFFIN IMBEDDING—

- A. Fixing.
- B. Dehydration.
- C. Clearing.
- D. Imbedding in paraffin.
- E. Molding block.
- F. Cutting.
- G. Method of numbering, registering, and storing.

CELLOIDIN IMBEDDING—

- A. Prepare a thin celloidin solution containing 4 per cent celloidin in equal parts of absolute alcohol and ether.
- B. Prepare a saturated solution in the same solvent.
- C. Transfer tissue from absolute alcohol to equal parts of alcohol and ether.
- D. From alcohol and ether to thin celloidin.
- E. From thin celloidin to thick celloidin.
- F. From thick celloidin to paper mold on cork.
- G. Into 70 per cent alcohol for three days to harden.
- H. Cut section with knife flooded with 70 per cent alcohol.
- I. Stain.
- J. Dehydrate.
- K. Clear.
- L. Mount.

METHOD OF MAKING FROZEN SECTIONS. (Wright.)

1. Place tissue not over 5 mm. thick in 10 per cent formalin for two or more hours.
2. Wash in water.
3. Freeze with CO₂ or ether.
4. Cut frozen sections.
5. Float sections in normal salt solution and then on slide.
6. Cover section with cigarette paper, then with blotter moistened with 95 per cent alcohol. Press. Remove papers.
7. Cover with absolute alcohol thirty seconds. Drain.
8. Flow over the section and adjacent parts of the slide a thin solution of celloidin in equal parts of absolute alcohol and ether. Drain off immediately. The celloidin should form a coating so thin as to be invisible.
9. Flood the slide with 95 per cent alcohol and then immerse in water. This hardens the film.
10. Stain.
11. Dehydrate.
12. Clear with oil organum.
13. Mount in Canada balsam.

STAINING TISSUES IN SECTIONS.

1. Hem-alum and eosin.
2. Delafield's hematoxylin.
3. Eosin and thionin.
4. Carmine stains.
5. Weigert's fibrin stain.
6. Jenner's stain.
7. Van Giesen for connective tissues.

STAINING BACTERIA IN SECTIONS.

1. Gram's method.
2. Eosin and thionin.
3. Thionin.
4. Methylene blue.

STUDY OF TUMORS.

Classification.

Cut sections and study :

1. Myomata.
2. Neuromata.
3. Angiomata :
 - a. Simple.
 - b. Cavernous.
4. Lymphangiomata :
 - a. Simple.
 - b. Cavernous.
5. Fibromata.
6. Myxomata.
7. Lipomata.
8. Chondromata.
9. Osteomata.
10. Gliomata.
11. Sarcomata :
 - a. Round celled.
 - b. Lympho-sarcoma.
 - c. Alveolar.
 - d. Spindle celled.
 - e. Melano sarcoma.
 - f. Osteoid.
 - g. Psammomata.
 - h. Myeloid.
 - i. Angio sarcoma.
 - j. Endothelioma.
12. Papillomata.
13. Adenomata.
14. Carcinomata :
 - a. Acinous.
 - b. Epitheliomata.
15. Cystomata.

ANIMAL INOCULATIONS—

1. Subcutaneous method :
 - a. Liquids.
 - b. Solids.
2. Intravenous method.
3. Inoculation into lymphatic system.
4. Inoculation into serous cavities :
 - a. Peritoneum.
 - b. Pleural cavity.
 - c. Subdural space.
5. Inoculation into anterior chamber of eye.

OBSERVATION OF ANIMALS AFTER INOCULATION—

1. Temperature.
2. Loss of weight.
3. Peculiar position in cage.
4. Loss of appetite.
5. Condition of coat.
6. Condition of secretions, etc.

POST-MORTEM EXAMINATION OF ANIMALS—

1. Cultures from the organs.
2. Cultures from the fluids.
3. Preparation and staining of tissues.

INCINERATION OF DEAD ANIMALS AFTER EXAMINATION—**MAKING COLLODIUM SACKS—**

Paris method.

Gelatin capsule method.

With glass support and gelatin (method of Grubbs & Francis).

BACILLUS COLI Migula, 1900. [*Bacterium coli commune* Escherich.]

- A. Distribution.
- B. Isolate from sewage.
- C. Characteristics :
 - 1. Motility.
 - 2. Gram's method.
 - 3. Nonliquefaction of gelatin.
 - 4. Indol.
 - 5. Gas production.
 - 6. Colonies, etc.
- D. Pathogenesis.
- E. Varieties.
- F. Preparation of its toxin.
- G. Pathogenicity.
- H. Study of blood and organs of infected animals.
- I. Significance in drinking water.
- J. Viability.

BACILLUS ANTHRACIS Koch, 1876.

- A. History.
- B. Distribution.
- C. Morphology:
 - 1. Involution forms.
 - 2. Asporogenous form.
- D. Staining.
- E. Cultural characteristics.
- F. Spores :
 - 1. Formation—
 - Relative to oxygen.
 - Relative to nutrient media.
 - Relative to temperature.
 - 2. Development into bacteria.
- G. Pathogenesis (mouse, guinea pig, and rabbit.)
 - a. Pneumonic.
 - b. Hemorrhagic.
 - c. Local.
- H. Examination of liver, blood, spleen, lungs, and other organs.
- I. Vaccination :
 - First vaccine—kills mice, not guinea pigs or rabbits.
 - Second vaccine—kills mice and guinea pigs, not rabbits.
 - Third vaccine—kills mice and guinea pigs and some rabbits.
- J. Disposal of anthrax carcasses :
 - a. Burial, isolated districts.
 - b. Burning.
 - c. Sulphuric acid.
 - d. Boiling.
- K. Human anthrax :
 - a. Malignant pustule.
 - b. Wool sorter's disease.
 - c. Intestinal form.
- L. Viability.
- M. Disinfection for anthrax.

BACTERIUM DIPHThERIAE (Löffler) Migula. [*Klebs-Löffler Bacillus*.]

- A. History.
- B. Morphology—relation to clinical types.
- C. Staining:
 - 1. Löffler.
 - 2. Neisser.
 - 3. Roux.
 - 4. Weigert's fibrin method for tissues.
 - 5. Gram's stain.
- D. Biologic characteristics.
- E. Pathogenesis:
 - 1. Guinea pig.
 - 2. Rabbit.
 - 3. Kitten.
- F. Toxin production.
- G. Antitoxin production:
 - 1. Standardization.
 - 2. Prophylactic use.
 - 3. Curative use.
- H. Obtain tubes and swabs from the health department for diagnosis.
 - I. Pseudo-diphtheria bacillus.
- K. Xerosis bacillus.
- L. Viability.
- M. Disinfection for diphtheria.

BACTERIA IN INFLAMMATION AND SUPPURATION—

Study :

- A. *Micrococcus aureus* (Rosenbach, 1884) Migula, 1900. [*Staphylococcus pyogenes aureus* Rosenbach, 1884.]
- B. *M. pyogenes* (Rosenbach, 1884) Migula, 1900. [*S. pyogenes albus* Rosenbach, 1884.]
- C. *M. citreus* (Passet, 1885) Migula, 1900. [*S. pyogenes citreus* Passet, 1885.]
- D. "*Staphylococcus epidermidis albus* Welch."
- E. *M. gonorrhææ* (Bumm, 1885) Flügge, 1886.
- F. *Streptococcus pyogenes* Rosenbach, 1884.
- G. "*Streptococcus erysipelatos* Rosenbach," 1884
- H. *Pseudomonas aeruginosa* (Schröter, 1872) Migula, 1900. [*Bacterium aeruginosum* Schröter, 1872 ; *Bacillus pyocyaneus* Gessard, 1882.]

Collect pus and inflammatory exudates from various sources, plate out and study bacteria found.

Study pus in smears on slides.

Ducrey's bacillus—Soft chancre.

Micrococcus melitensis Bruce, 1893.

THE TYPHOID GROUP—

Study :

- A. *Bacillus typhosus* Migula, 1900.
- B. *Bacillus dysenteriae* Shiga, 1898 [not Migula, 1900].
- C. *Bacillus enteritidis* Gaertner, 1888.
- D. Para-colon bacillus.
- E. Para-typhoid bacillus.
- F. *Bacillus murium* Migula, 1900. [*Bacillus typhi murium* Löffler, 1893.]
- G. Bacillus of Danyz's virus.
- H. *Bacillus psitticosis*.
- I. *Bacillus icteroides* Sanarelli, 1897.
- J. *Bacillus coli* Migula, 1900. [*Bacterium coli commune* Escherich.]
- K. Methods of introduction into the system.
- L. Channels of elimination.
- M. Viability.
- N. Disinfection.
- O. Relation to the water supply.
- P. Relation to flies and other insects.
- Q. Widal reaction.—
 - a. With hanging drop.
 - b. In pipettes.

THE TUBERCLE GROUP—

A. *Bacillus tuberculosis* Koch, 1882.

1. Human. Isolate in pure culture from sputum.
2. Bovine.
3. Avian.

Tuberculin—

Production.

Uses:

1. Diagnostic.
2. Therapeutic.

B. Acid proof organisms, resembling *B. tuberculosis*:

1. Moller's *gram* bacillus. Isolate from timothy.
2. Kahnawitch's butter bacillus.
3. Karlinaky's nasal secretion organism.
4. *Smegma* bacillus.

C. *Bacillus leprae* Hansen.

D. *Bacillus mallei* (Löffler, 1893) Migula, 1900:

Mallein.

Strain method in male guinea pig.

E. Actinomycosis.

F. Mycetoma, Madura foot.

G. Pathogenic streptothrices.

THE VIBRIO GROUP—

- A. *Microspira comma* Schröter, 1886. [Koch's comma bacillus of Asiatic cholera.]
- B. *Microspira Metchnikovi* (Gamaleia, 1888) Migula, 1900.
- C. *Microspira Finkleri* Schröter.
- D. *Vibrio Denecke*.
- E. *Microspira aquatilis* (Günther, 1892) Migula, 1900.
 - Pfeiffer's phenomenon.
 - Widal reaction.
 - Immunity.
 - Haffkine's prophylactic.
 - Viability.
 - Disinfection for cholera.

RELAPSING FEVER.

BACILLUS PESTIS Kitasato & Yersin, 1894.**A. Study—morphology, staining and cultural characteristics :**

Viability.

Channels of entrance.

Channels of elimination.

Pathogenicity—guinea pigs, rabbits, rats, mice.

Diagnosis :

Yersin serum—

Method of manufacture and standardization.

Prophylactic use.

Curative use.

Haffkine prophylactic—

Methods of manufacture.

Uses.

B. Disinfection.**C. Quarantine.****D. Relation of rats and other mammals to spread of plague.****E. Relation of fleas, flies, and insects to spread of plague.**

SEPTICEMIC GROUP—

- A. Mouse septicemia.
- B. Rouget du porc.
- C. Chicken cholera.
- D. Swine plague.
- E. Ferret, duck, buffalo, and other plagues.
- F. *Bacillus pestis* Kitasato & Yersin, 1894.
- G. *Bacterium pneumoniæ* (Weichselbaum, 1886) Migula, 1900. [*Diplococcus pneumoniæ* Weichselbaum, 1886.]
- H. *Bacterium pneumonicum* Migula, 1900. [*Pneumonicoccus* Friedländer, 1882.]
- I. *Bacterium influenzae* Lehmann & Neumann, 1896.

ANAEROBIC TECHNIQUE—**A. Kipps' hydrogen apparatus :**

1. Mechanics.
2. Chemicals.

B. Simple hydrogen generators.**C. Solutions for purifying gas :**

1. Silver nitrate for chlorine.
2. Potassium permanganate for oxygen.
3. Lead acetate for sulphides.

D. Media best suited for anaerobes :

1. Freshly prepared media.
2. Glucose media. .

E. Special methods :

1. Fraenkel's method.
2. Esmarch's method.
3. Liborius' method.
4. Buchner's method.
5. Novy's jars.
6. Wright's method.
7. Pasteur Institute method.
8. Vignal's tubes.
9. Full tubes of bouillon.
10. Deep stabs.
11. Oil method.

ANAËROBES—

A. *Bacillus tetani*, its toxin and antitoxin.

B. *Bacillus œdematis* of malignant edema.

C. *Bacillus Chauvæi* Arloing, Cornevin & Thomas, of symptomatic anthrax :

Vaccine—

1. Manufacture.

2. Use.

D. *Bacillus Welchii* Migula, 1900. [*Bacillus aerogenes capsulatus* Welch & Nuttall, 1892.]
Disinfection for above.

PATHOGENIC MOLDS—

*Ring worm:**Microsporon Audouini* (Gruby).*Trichophyton*—1. *T. endothrix*.2. *T. ectothrix*.

Hair lesions.

Pure cultures.

*Favus:**Achorion Schoenleinii* Remak.

In the hair and scale.

In pure culture.

YEASTS—

Saccharomyces cerevisiæ Meyen (beer yeast) in pure cultures:

Effect on saccharine media.

Budding.

Red yeast—*S. glutinis*.

Black yeast—*S. niger*.

Saccharomyces albicans (Robin) Reess, 1877 [*Oidium albicans* Robin] thrush, in pure culture.

Pathogenic yeasts.

EXAMINATION OF URINE—

Note:

1. Odor.
2. Color.
3. Deposit. Filtration of specimen.
4. Specific gravity.
5. Reaction.
6. Quantity in twenty-four hours.
7. Albumen—

Tests for albumen; quantitative and qualitative:

 - a. Heat (phosphates).
 - b. Nitric acid, in test tube and in glass.
 - c. Ferro-cyanide potash; acetic acid.
 - d. Picric acid, Esbach's albumenometer.
8. Sugar—

Test for sugar:

 - a. Fehling's solution.
 - b. Bismuth.
 - c. Quantitative estimation with yeast and with Fehling's solution.
9. Estimation of urea.
10. Bile, test for.
11. Microscopic examination of sediment for bacteria, casts, crystals, blood, etc.,
Schistosoma hæmatobium (Bilharz, 1852) Weinland, 1858.
12. Test for chlorides.
13. Diazo reaction.
14. Bacteriologic examination of urine.
15. Disinfection of urine.

BACTERIOLOGIC AND MICROSCOPIC EXAMINATION OF FECES—

1. Microscopic :

a. Normal constituents.

b. Intestinal parasites and their eggs—

Ascaris lumbricoides (Linnaeus, 1758).*Oxyuris vermicularis* (Linnaeus, 1758) Bremser, 1819.*Trichuris trichiura* (Linnaeus, 1771).*Uncinaria duodenalis* (Dubini, 1843).*Uncinaria americana* Stiles, 1902.*Tænia saginata* Goëze, 1782.*Tænia solium* Linnaeus, 1758.*Dibothriocephalus latus* (Linnaeus, 1758).*Amœba coli* Lœsch, 1875.

2. Bacteriologic :

a. Normal flora.

b. Pathogenic—

*Bacillus dysenteriz.**Bacillus tuberculosis.**Bacillus typhosus.**Microspira comma.*

3. Disinfection of feces.

BACTERIOLOGIC AND MICROSCOPIC EXAMINATION OF—

Sputum, nasal and other mucous secretions :

Tubercle bacilli.

Pneumococci.

Influenza bacterium.

Disinfection of sputum.

BLOOD.

Technique of:

1. Collecting blood for microscopic examination.
2. Collecting blood for bacteriologic examination—
 - a. Small amount.
 - b. Large quantities.
 - Of man.
 - Of other mammals.
 - Of birds.
 - Of reptiles.
3. Observe circulation of blood under microscope.

Mesentery of frog.
4. Estimate alkalinity.
5. Estimate specific gravity of blood with mixture of chloroform and benzol.
6. Examine fresh blood in hanging drop.
7. Examine fresh blood between slide and cover slip.
8. Study of blood films :
 - a. Method with cover glass.
 - b. Method with special rod.
 - c. Method with cigarette paper.
9. Fixation of blood films :
 - a. Heat, 150° C. for about five minutes.
 - b. Alcohol and ether.
 - c. Formalin.
 - d. Formaldehyd.
 - e. Acetic acid bichlorid.
10. Staining.
 - a. Ehrlich-Biondi-tri-acid stain.
 - b. Jenner's stain.
 - c. Romanowsky's stain.
 - d. Goldhorn's fluid.
 - e. Eosin and methylen blue.
 - f. Thionin and eosin.
11. Counting red corpuscles.

Thoma-Zeiss hemocytometer.
12. Estimating the number of leucocytes :
 - a. With the Thoma-Zeiss hemocytometer.
 - b. Comparative count of—
 - a. Small lymphocytes.
 - b. Large lymphocytes and transitional forms.
 - c. Polymorphonuclear neutrophiles.
 - d. Eosinophiles.
 - e. Mast cells.
13. Estimating the amount of hemoglobin :

With—

 - a. Dare's hemoglobinometer.
 - b. Oliver's hemoglobinometer.
 - c. Gower's hemoglobinometer.

BACTERIA IN THE AIR—**1. Number :**

Sewer air.

Expired air.

Sea air.

City air.

Country air.

Air in houses, hospitals, etc.—

Relation to the sun.

Relation to habitation.

Relation to winds and traffic.

Relation to altitude.

Pasteur's method.

Miquel's method.

Aeroscopes.

Frankland's method.

Petri's method.

Hesse's method.

Straus' method.

2. Varieties.**3. Estimation of ammonia in the air.****4. Estimation of carbon dioxid in the air**

BACTERIA IN WATER—

1. Count number per 1,000 c. c. :
 - a. In tap water.
 - b. In river water.
 - c. In spring water.
 - d. In rain water.
 - e. In ice.
2. Varieties.
3. Collecting samples.
4. Estimation of organic matter—
 - a. Free ammonia.
 - b. Albuminoid ammonia.
5. Estimation of nitrates and nitrites.
6. Estimation of chlorids.
7. Purification of water :
 - Heat.
 - Filters—
 - a. Household filters.
 - b. Field and camp filters.
 - c. Sand filters—
 - Alum.
 - Hyperoxid of chlorin.
 - Ozone.
 - Permanganate of potash.

BACTERIA IN SOIL—**1. Number.****2. Varieties.****Nitrification.**

PURIFICATION AND DISPOSAL OF SEWAGE.**1. Dilution.**

This method is allowable for cities situated on the sea or on rivers whose flow is very large as compared with the sewage (100:1) and the unfiltered water of which is not used as a water supply.

2. Sewage farming.**3. Chemical precipitation.****4. Intermittent filtration.**

Needs sandy soil.

5. Septic tank.**6. Contact bed treatment.****7. Continuous filtration :**

a. Scott-Moncrieff system.

b. The Ducat.

c. Whitaker & Bryant.

d. F. Stoddart.

e. G. Salford.

MILK—

1. Histology.
2. Bacteria in milk :
 - Number.
 - Origin.
 - Varieties.
 - Diseases conveyed by milk.
3. Fermentation—
 - Lactic acid.
 - Butyric acid.
 - Sweet curdling.
 - Abnormal.
4. Pasteurization :
 - Theory and practical uses.
 - Changes produced in milk.
5. Count and study colonies in fresh milk and milk bought in open market.
 - Pasteurize, then count and study colonies.
6. Sterilization of milk by heat ; practical application and changes produced in milk.
7. Milk preservatives.

PRINCIPLES AND PRACTICE OF DISINFECTION AND STERILIZATION.

Physical agents.

1. Sunlight—

Expose spores of anthrax, subtilis, etc.

Expose nonspore-bearing organisms as typhoid, diphtheria, etc., to the sunlight in thin films and for penetration. Note intensity of the light, temperature, dryness, and other conditions. Plant in bouillon.

2. Electricity—

Expose slips and cultures to various currents.

Expose to electric light.

Expose to X-rays.

3. Dryness and dry heat—

Expose slips, or threads with spore-bearing and nonspore-bearing organisms to :

a. Dryness at room conditions.

b. Dry in incubator at 37° C.

c. Dry over sulphuric acid.

d. Dry heat at 60° C.,

100° C.,

150° C.,

for variable times.

e. Expose paper slips saturated with—

a. Decomposing urine,

b. Sewage,

c. Garden earth,

to 150° C. for one hour to test power of sterilization of this temperature.

Hot-air sterilizer.

NOTE.—Plant in bouillon and keep one week for growth.

5. Moist heat—hot and boiling water and steam :

Expose typhoid, diphtheria, and other nonspore-bearing organisms to 50°, 60°, 70°, 80°, and 100° C. moist heat for variable times ; plant in bouillon.

Expose anthrax, subtilis, and other spores to 70°, 80°, 90°, and 100° C. moist heat for variable times ; plant in bouillon.

6. Theory, mechanics, and practical application of steam in—

a. Koch steamer.

b. Arnold steam sterilizer.

c. Autoclave (steam under pressure).

d. Steam chamber (steam under pressure).

Make practical tests in each.

TESTING OF ANTISEPTICS—

If the substance belongs to the Cresol group, prepare 1 per cent, 2 per cent, 3 per cent, 5 per cent, 7 per cent, 9 per cent, 10 per cent solutions of the substance in a set of bouillon tubes.

If the substance belongs to the metallic salts, make $\frac{1}{5000}$, $\frac{1}{3000}$, $\frac{1}{2000}$, $\frac{1}{1000}$, $\frac{1}{500}$, etc., solutions in a set of bouillon tubes.

Inoculate with :

- a. Decomposing urine.
- b. Sewage.
- c. Garden earth.
- d. Typhoid.
- e. Pus cocci.
- f. Anthrax spores, etc.

Incubate and make report on the following plan :

Name of substance.—Strength of solution.	Minutes.							
	1.	2.	3.	5.	10.	15.	20.	30.
Sewage	—	—	—
Garden earth	—	+	+
Decomposing urine.....	+	+	+

— means no growth, + means growth.

TESTING GERMICIDES—

Saturate silk threads,
paper slips,
glass slips, etc., with
subtilis spores,
anthrax spores,
typhoid bacillus,
diphtheria bacillus,
pus cocci, etc.

Expose to the solutions of germicide in varying dilutions and for varying times at constant temperature, and at the same dilution at varying temperatures.

Wash or neutralize the germicide.

Plant in bouillon.

Incubate.

Report growth upon similar table to antiseptics.

Test the following upon the above plan—

- A. Bichloride of mercury.
- A. Carbolic acid and cresols.
- C. Lime.
- D. Copper and iron sulphates.
- E. Formalin.

GASEOUS DISINFECTION—**A. Formaldehyd :**

- a.* Chemistry and physics.
- b.* Sheet method.
- c.* Generator—
 - 1. Kuhn lamp.
- d.* Regenerators without pressure—
 - 1. Trenner-Lee.
 - 2. Lentz.
- e.* Regenerators with pressure—
 - 1. Autoclave.
- f.* Heating paraform.
- g.* Spraying.

B. Sulphur dioxid (SO_2):

- a.* Liquid sulphur dioxid.
- b.* Burning sulphur, pot method.
- c.* Sulphur furnace.

C. Hydrocyanic acid gas.**D. Chlorin gas.****E. Ozone and oxygen.**

INSECTICIDES—

- A. Tobacco smoke.
- B. Pyrethrum.
- C. Hydrocyanic acid.
- D. Formaldehyd gas.
- E. Sulphur dioxide.
- F. Coal oil.
- G. Arsenical compounds.
- H. Phosphorus.
- I. Cobalt salts.
- J. Carbon bisulphid for mosquitoes, adults, pupæ, and larvæ :
 - Ants,
 - Mosquitoes,
 - Roaches,
 - Fleas,
 - Flies, etc.

1. THE DISINFECTION OF HOUSES, SHIPS, AND OBJECTS.
2. THE DISINFECTION AND PROPHYLAXIS AGAINST THE COMMUNICABLE DISEASES, ESPECIALLY:
 - a. Yellow fever.
 - b. Cholera.
 - c. Plague.
 - d. Smallpox.
 - e. Tuberculosis.
 - f. Typhoid fever.
 - g. Malaria.
 - h. Dysentery.
 - i. Diphtheria.
 - j. Pneumonia.
 - k. Leprosy.
 - l. Anthrax.
 - m. Tetanus.
 - n. Dengue.
 - o. Relapsing fever.
 - p. Typhus fever.
 - q. Scarlet fever.
 - r. Measles.

Including channels of introduction and elimination.

Viability.

Disinfection.

Prophylaxis.

Management of epidemics.

Quarantine.

RABIES—

Study in dogs.

Study in rabbits.

Prepare spinal cords for preventive inoculation.

Principles and methods of Pasteur treatment.

VACCINATION (*vaccinia*)—

- A.** History.
- B.** Methods of production :
 Vaccine lymph.
 Vaccine pulp—
 Dry points.
 Glycerinated pulp.
- C.** Technique of vaccination.
- D.** Immunity produced.
- E.** Bacteriologic and microscopic study of vaccine matter.
- F.** Bodies in epithelial cells—cornea of rabbits.

Examination of vaccine—

- G.** Note the name of maker, kind of virus, etc.
- H.** Count the number of colonies upon :
 - 1. Three glycerinated tubes upon agar.
 - 2. Three glycerinated tubes or points upon gelatin.
 - 3. Three dry points upon agar.
 - 4. Three dry points upon gelatin.
- I.** Study the character of the organisms found upon media and animals.
- J.** Plant the entire contents of 6 tubes in bouillon, grow anaërobically and study the characters of the growth upon media and by inoculation in animals.
- K.** Study the dry points as in J.
- L.** Inoculate the contents of 12 tubes and 12 points directly into mice, rats, and guinea pigs.
- M.** Keep full notes of all results.
- N.** Visit to the vaccine farm for practical study of manufacture of vaccine commercially.

MALARIA—

1. Diagnosis.
2. Blood technique.
3. Relation to mosquito :
 - a. Tertian.
 - b. Quartan.
 - c. Aestivo-autumnal.

Mosquitoes—

Anatomy—dissections fresh specimens.

Sections in paraffin.

Development and raising.

Classification.

Diagnosis, especially :

- a. *Culex*.
- b. *Anopheles*.
- c. *Stegomyia*.

A. Malaria—the *Anopheles* :

Study *Anopheles* one each day after biting a malarial case.

Tertian.

Quartan.

Estivo-autumnal.

B. Yellow fever and the mosquito.**C. Filaria and the mosquito.**

INSECTS AS FACTORS IN TRANSMITTING DISEASE—

The mosquito.

The fly.

The ant.

The flea.

The bedbug.

The roach.

The tick, etc.

Texas fever.

Spotted fever of Bitter Root Valley, Mont.

TRYPANOSOMES—

Inoculate rat with *Trypanosoma Lewisi*.

Dourine.

Surra.

Nagana.

Mal de caderas.

FILARIAL EMBRYOS (“*Filaria sanguinis hominis*”) in the blood.

Filaria Bancrofti Cobbold, 1877.

Filaria diurna Manson, 1891.

Filaria perstans Manson, 1891.

Filaria Demarquayii Manson, 1895.

Dracunculus medinensis (Linnaeus, 1758). [Guinea Worm.]

FERMENTS—

Organized.

Unorganized—enzymes.

A. *Amylolytic*:

Ptyalin.

Diastase.

Amylopsin.

Convert starch and glycogen into sugar.

B. *Proteolytic*:

Pepsin.

Trypsin.

Bacterial peptonizing enzymes.

Convert proteids into peptones.

C. *Steatolytic*—steapsin.

Split fats into fatty acids and glycerin.

D. *Invertin*—in intestinal juice and yeast cells.

Convert cane sugar, maltose, lactose into glucose.

E. *Coagulation*:

Rennet.

Fibrin ferment.

Convert soluble into insoluble ferments.

F. *Alcoholic ferments*:

Saccharomyces.

Converts carbohydrates into alcohol, carbon dioxide, etc.

Fermentation in—

Wine.

Beer.

Vinegar, acetic.

Milk:

Lactic.

Butyric.

Koumys.

Kefir.

IMMUNITY—**Natural.****Artificial.****Active.****Passive.****Acquired.****Serum therapy—****Anti-infectious sera.****Antitoxic sera.****Prophylactic sera.****Curative sera.****Ehrlich's side chain theory.****Phagocytosis.****Preventive vaccines—****Vaccinia (smallpox).****Rabies.****Anthrax.****Symptomatic anthrax.****Swine plague.****Hog cholera.****Rouget du porc.****Plague.****Typhoid fever.****Cholera.****Tetanus.****Diphtheria.**

**TRANSPORTATION OF DISEASED TISSUES AND BACTERIOLOGIC SPECI-
MENS, ETC., BY MAIL AND EXPRESS.**

The Regulations of the Post-Office Department, Order No. 176, March 2, 1900.

Mailing cases.

Methods of sending dry specimens.

Methods of sending moist specimens.

Methods of preparing and forwarding samples for bacteriologic study.

Methods of preparing and forwarding samples for histologic study.



TREASURY DEPARTMENT.
Public Health and Marine-Hospital Service of the United States.
WALTER WYMAN, Surgeon-General.

HYGIENIC LABORATORY.—BULLETIN No. 9.

M. J. ROSENAU, Director.

SEPTEMBER, 1902.

PRESENCE OF TETANUS
IN
COMMERCIAL GELATIN

BY

JOHN F. ANDERSON.



WASHINGTON:
GOVERNMENT PRINTING OFFICE
1902.

PRESENCE OF TETANUS IN COMMERCIAL GELATIN.

[BY JOHN F. ANDERSON, ASSISTANT SURGEON, UNITED STATES PUBLIC HEALTH
AND MARINE-HOSPITAL SERVICE.]

Gelatin injections are now used rather extensively, in Europe especially, as a means of treatment for aneurism and as a direct and prophylactic hemostatic. It is generally used in a 2 per cent solution in water, boiled immediately before use, and, as a hemostatic, injected subcutaneously. There seems a great variation in the amount of solution used, some using 200 c. c. and others as high as 1,000 c. c.

Gelatin is a substance obtained from the nitrogenous portions of bone, hides, connective tissue, etc. The collagen when boiled is changed to gelatin. Glue is impure gelatin to which has been added some preservative and some substance to increase its power of adhesion. Bone and hide gelatin are the two principal kinds of gelatin. Isinglass is gelatin made from the bladders of sturgeons. According to Whipple (5), the general process of manufacturing hide gelatin is as follows :

The hide scraps are first macerated and subjected to the action of a solution of lime or caustic soda in pits for two or three weeks. This dissolves most of the blood and saponifies the fats. The excess of lime or soda is then largely removed by washing and the solution steamed to dissolve the gelatin, but an excess of heat is avoided. Sulphurous acid is used to bleach the gelatin. When of sufficient strength, the gelatin is allowed to harden in molds or on slabs, and is ultimately dried in sheets on wire nets. Bone gelatin is made in a somewhat similar manner. The bones are crushed, boiled, treated with hydrochloric acid, and the gelatin is dissolved as before, washed, bleached, and dried in sheets. The process requires a number of weeks.

According to the National Dispensatory, gelatin is made by boiling in water for a long time bone cartilage, animal skin, tendons, and ligaments. These substances become soluble in the boiling water, and when the mass is allowed to cool, form a jelly. The animal tissues are placed upon a sieve or perforated diaphragm some distance from the bottom of the boiler. When the solution has become sufficiently saturated it is drawn off and allowed to cool, and the jelly is then cut into thin sheets and placed on wire nettings to dry. While so exposed to the air for drying it could easily become infected with tetanus spores, as the gelatin is often manufactured in conjunction with the other procedures that go on in a large meat-packing house.

Kuhn (1) reported a case of tetanus following the injection of gelatin in a hemophilic.

Gerulanos, Georgi, and Lorenzo (2) report four cases of tetanus following the injection of gelatin for the purpose of arresting hemorrhage

after operations such as for subphrenic abscess and for disease of the urinary tract. In one of these last four cases the organism was obtained from the seat of injection.

Drs. Hochhalt and Herezel (3) report a case of tetanus on the seventh day after an injection of 2 per cent gelatin following splenectomy.

Dr. Laszlo Deutsch (3) studied bacteriologically the gelatin used in the last-named case, and found that after being boiled for five minutes and then grown anaerobically it contained an anaerobic, spore-bearing bacillus. He advises that gelatin, to be used as a hemostatic, be inoculated with bacillus subtilis before sterilization, and, as this organism is more resistant to heat than that of tetanus, if there is no growth of subtilis the gelatin can be used without fear of tetanus.

Drs. Levy and Brun (4), of Strasbourg, examined six samples of gelatin and in four found tetanus bacilli. They found that the spores of the tetanus bacilli which they isolated were able to resist streaming steam for a longer period than eight minutes.

Six different makes of gelatin were purchased in the open market and, with the stock laboratory gelatin, an investigation was begun with regard to the presence of tetanus in the same. Ten grams of each sample of gelatin were placed in 100 c. c. of glucose bouillon and heated at 50° C. until dissolved, then rendered slightly alkaline to litmus and heated to 80° C. for ten minutes to endeavor to kill all nonspore-bearing organisms; the flasks at the end of ten minutes were quickly placed in a Novy jar and boiled in vacuo to expel all of the air; the jars were then placed in the incubator and kept at 37° C. for one week. On the seventh day the flasks were removed from the jars. Three of the flasks remained sterile; of the other four, three showed an end-spore bearing rod, the spores of two of them being oval, and the third round with a very fine rod; this last stained by Gram.

The same day .5 c. c. of each culture was injected subcutaneously into white rats, but beyond making them very sensitive to sudden noises for one or two days, no results were noted. Two days later .5 c. c. of the culture containing the round, end-spore rod was put into a white mouse. The mouse was found dead the next morning. Bouillon tubes were inoculated from the site of inoculation and with the heart's blood, but both remained sterile. Two drops of this culture, which we will call No. 1, were put into a tube of bouillon, heated to 80° C. for five minutes, and from this glucose-agar plates were made and incubated for seven days at 37° C. in a Novy jar. At the end of a week two of the plates showed deep, oval colonies; stained smears showed these to be a fine rod with a round end-spore.

A flask and three tubes of *freshly* made bouillon were inoculated from the plates; the flask was heated to 80° C. for ten minutes; the tubes were not heated after inoculation. Flask and tubes were grown in a Novy jar as before. One drop of the bouillon growth from one of the tubes was injected into the right side of a mouse; mouse died next

morning with signs of tetanus, such as stiff tail and retraction to right side. Smears made from the site of inoculation showed the presence of a thin rod with a round end-spore. Decreasing doses were put into white mice on successive days until finally it was found that .000022 c. c. would kill a mouse with typical symptoms of tetanus in four days, with an incubation period of about thirty-six hours.

SUMMARY AND CONCLUSIONS.

Seven samples of gelatin examined; one showed tetanus spores.

Two samples showed an oval end-spore rod, whose identity was not proved, but in stained specimens it would be hard to distinguish from tetanus, if indeed not tetanus with diminished virulence.

In tetanus investigations it is important to use *freshly* made bouillon, as the organism is apt not to germinate in bouillon over ten days old.

The thermal death point of the organism isolated was found to be between twenty and thirty seconds at 100° C.

It is important, therefore, that gelatin to be used for injections should be boiled at least ten minutes on account of the variability of the thermal death point in different species of tetanus. Whether this amount heating impairs in any way the hemostatic power of gelatin has not been settled, but in case it does it is believed that the danger from tetanus more than overbalances its therapeutic value.

It is suggested that when, as in hospitals, there is likelihood of gelatin injections being used for hemostatic purposes the gelatin solution be sterilized by the fractional method on three successive days and kept ready for use in sterile containers.

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TREASURY DEPARTMENT.

Public Health and Marine-Hospital Service of the United States.

WALTER WYMAN, Surgeon-General.

HYGIENIC LABORATORY.—BULLETIN No. 10.

M. J. ROSENAU, Director.

February, 1903.

REPORT

UPON THE

PREVALENCE AND GEOGRAPHIC DISTRIBUTION OF HOOKWORM DISEASE

(*Uncinariasis or Anchylostomiasis*)

IN THE

UNITED STATES.

BY

CH. WARDELL STILES, Ph. D.

Chief of Division of Zoology.



WASHINGTON:
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1903.

ORGANIZATION OF HYGIENIC LABORATORY.

WALTER WYMAN, *Surgeon-General*,
U. S. Public Health and Marine-Hospital Service.

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SUMMARY

Convinced from theoretical deductions that hookworm disease (uncinariasis) must be more or less common in the South, a trip was made from Washington, D. C., to Ocala, Fla., stopping at penitentiaries, mines, farms, asylums, schools, and factories, and the fact was established that the chief anemia of the Southern rural sand districts is due to uncinariasis, while clay districts and cities are not favorable to the development of this disease.

In the Old World, hookworm disease was probably known to the Egyptians nearly three thousand five hundred years ago, but its cause was not understood until about the middle of the nineteenth century, when it was shown to be due to an intestinal parasite, *Agchylostoma duodenale*. Until 1893 no authentic cases of this disease were recognized as such in the United States, but between 1893 and 1902 about 35 cases were diagnosed. In 1902 it was shown that a distinct hookworm, *Uncinaria americana*, infests man in this country, and this indicated very strongly that the disease must be present although not generally recognized. It is now established that in addition to the few cases of Old World hookworm disease imported into the United States we have in the South an endemic uncinariasis due to a distinct cause, *Uncinaria americana*. This disease has been known for years in the South and can be traced in medical writings as far back as 1808, but its nature was not understood. Some cases have been confused with malaria, others have been attributed to dirt-eating.

The hookworms are about half an inch long. They live in the small intestine, where they suck blood, produce minute hemorrhages, and in all probability also produce a substance which acts as a poison. They lay eggs which can not develop to maturity in the intestine. These ova escape with the feces and hatch in about twenty-four hours; the young worm sheds its skin twice and then is ready to infect man. Infection takes place through the mouth, either by the hands soiled with larvæ or by infected food. Infection through the drinking water may possibly occur. Finally, the larvæ may enter the body through the skin and eventually reach the small intestine.

Patients may be divided into light cases, in which the symptoms are very obscure; medium cases, in which the anemia is more or less marked, and severe cases, represented by the dwarfed, edematous, anemic dirt-eater. Infection occurs chiefly in rural sand districts. Above the frost line the symptoms are more severe in summer than in winter, and whites appear to be more severely affected than negroes. Persons who come in contact with damp earth are more commonly infected than others, so that the disease is found chiefly among farmers, miners, and brickmakers. Severe cases are more common in women and children than in men over 25 years of age. Uncinariasis is a disease which occurs in groups of cases, and if one case is found in a family the chances are that other members of the same family are infected.

The testimony of patients severely infected is unreliable. Recalling that any one or more symptoms may be absent or subject to variation, it may be noted that the period of incubation (at least before the malady can be diagnosed by finding the eggs) is from four to ten weeks. Stages are not necessarily distinctly defined, but are described as (1) stage of purely local symptoms, corresponding to the light cases; (2) stage of simple anemia, corresponding to the medium cases; and (3) dropsical stage, corresponding more or less to the severe cases. The duration of the disease after isolation from the source of infection has been traced for six years and seven

months; how much longer infection will last is not established. If a patient is subject to cumulative infection, the disease may last five, ten, or even fifteen years, and in case of light infection perhaps longer.

External appearance.—In extreme cases there is a general lack of development; skin waxy white to yellow or tan; hair is found on the head, but is more or less absent from the body; breasts are undeveloped; nails white; external genitalia more or less rudimentary; face anxious, may be bloated; conjunctivæ pale; eyes more or less dry, pupil dilates readily; membranes pale according to the anemia; teeth often irregular; tongue frequently marked with purple or brown spots; cervical pulsations prominent; thorax emaciated; heart beats often visible; abdomen frequently with "pot belly;" extremities emaciated, frequently edematous, and with wounds or ulcers of long standing.

Urine 1010 to 1015; in advanced cases albumin without casts; acid or alkaline.

Feces reddish brown, contain eggs, and may contain blood.

Circulatory system.—Anemia pronounced, according to degree and duration of infection; blood watery, with decreased red blood corpuscles and with eosinophilia; "heart disease" very commonly complained of; hemic murmurs present; pulse 80 to 132 per minute.

Temperature.—Subnormal, normal, or to 101° or 102° F.

Respiratory system.—Breathing may be difficult, slow, or increased to as high as 30.

Muscular system.—Emaciation and great physical weakness.

Digestive system.—Appetite poor to ravenous; abnormal appetite often developed for pickles, lemons, salt, coffee, sand, clay, etc.; pain in epigastrium; constipation or diarrhea.

Nervous system.—Headache, dizziness, nervousness, mental lassitude, and stupidity.

Genital system.—Menstruation irregular or absent; if present, it occurs chiefly in winter; there is a marked tendency to abortion.

Diagnosis.—The safest plan is to make a microscopic examination of the feces to find the eggs; or, if feces are placed on white blotting paper, a blood-like stain will be noticed.

Treatment.—Thymol, or male fern (or ? calomel); iron, and good food.

Prognosis.—Good, if patient is not too far gone at time of treatment.

Lethality.—Not yet determined.

Prevention.—Treat all cases found and dispose of feces.

Economically, uncinariasis is very important. It keeps children from school, decreases capacity for both physical and mental labor, and is one of the most important factors in determining the present condition of the poorer whites of the sand and pine districts of the South.

The disease is carried from the farms to the cotton mills by the mill hands, but does not spread much in the mills; nevertheless, it causes a considerable amount of anemia among the operatives.

REPORT UPON THE PREVALENCE AND GEOGRAPHIC DISTRIBUTION OF HOOKWORM DISEASE (UNCINARIASIS OR ANCHYLOSTOMIASIS) IN THE UNITED STATES.

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INTRODUCTION.

Thoroughly convinced from theoretical zoologic considerations, especially of a faunistic nature, that uncinariasis must be a more or less common disease in the Southern portion of the United States, I requested instructions from Surgeon-General Wyman to study the subject in a field investigation. The desired authorization was received and the results of the work are contained in this paper.

DEFINITION.

Uncinariasis is a specific zooparasitic disease found especially in tropical and subtropical sand areas, and caused by hookworms (genus *Uncinaria*) which inhabit the small intestine. Its chief symptoms are: Anemia, with the circulatory symptoms found in all extreme anemias, namely, dizziness, palpitation, hemic murmurs; great weakness, in some cases with considerable emaciation; colicky pains in the abdomen; perverted appetite, such as "dirt-eating;" constipation or diarrhea, stools sometimes brownish or bloody; nausea; edema. The only positive diagnosis is by finding the parasite or its eggs in the stools. It may affect any class of patients, but is more frequent in persons whose daily life brings them in contact with damp earth (children, farmers, miners, brickmakers, excavators, etc.).

TERMINOLOGY.

The disease now under discussion is known by a number of different names, but uncinariasis^a should be adopted as the more correct technical designation. Among the names frequently applied to it, the

^aLooss (1902) has recently attempted to suppress the term "uncinariasis" in favor of anchylostomiasis, his view being that the genus *Agchylostoma* is distinct from *Uncinaria*. His suggestion does not help matters much at present. Even if the zoological genera are recognized as distinct, uncinariasis would still exist in man, while among animals it would be still more common than anchylostomiasis. Further, the two genera would probably have to be united in a subfamily, which could then be called "Uncinariinæ," and uncinariasis could then signify any infection of any

following may be mentioned in particular: Anchylostomiasis, ankylostomiasis, brickmakers' anemia, Egyptian chlorosis, miners' anemia, miners' cachexia, tunnel anemia, St. Gothard tunnel disease, tropical chlorosis, hookworm disease, and tunnel disease. (See also pp. 31, 32, 96.)

HISTORICAL REVIEW.

In order to understand the exact status of the subject of uncinariasis, it will be well to take a brief historical review of hookworms in general, hookworm disease in general, and hookworm disease in the United States.

BRIEF REVIEW OF HOOKWORMS.

It is quite probable that the ancient Egyptians, nearly thirty-five hundred years ago, were acquainted with the parasites which we now call hookworms. From a zoological standpoint, however, the first hookworm known to science was a parasite in the intestine of the common badger (*Meles taxus*) of Europe, described by Gœze, a German clergyman, in 1782. Gœze called the parasite "der Haarrundwurm" (the hair round worm), and gave to it the Latin name *Ascaris criniformis*. Although he placed this species in the same genus with the ordinary eelworm, *Ascaris lumbricoides*, he intimated that it represented a distinct genus. One of the anatomical characters which Gœze noticed was a membranous expansion on the tail of the male, and in this he saw two finger- or ray-like structures which he interpreted as "hooks" (see caudal rays in figs. 1, 15).

In 1789 Frœlich found a similar worm in the common fox (*Canis vulpes* or *Vulpes vulpes*) of Europe. He noticed the same membranous expansion and "two hooks with many points" on the end of the tail. On account of this character he adopted the vernacular name "Haakenwurm" (hookworm), and proposed the generic name *Uncinaria* for the new genus which he established.

It is now known that the membranous expansion is the caudal bursa, found in all members of the family Strongylidæ, while the so-called "hooks" represent the "rays" or "ribs" which support the bursa (see fig. 1). In the early part of the nineteenth century several other species of hookworms were described as parasitic in various animals, and they were united generically with the "colic worms" (strongyles) of horses.

animal with any member of this subfamily. In case the term "anchylostomiasis" is adopted, which of the many spellings should be recognized? Adopting uncinariasis relieves us of the necessity of discussing that point, and further gives to the name of the disease the same orthography in several different languages. The case at hand gives rise to the question whether it is not inadvisable to name diseases after the zoological names of the parasites, at least during the transitional stage of zoological nomenclature. As a matter of fact it is the function of the medical profession, not that of the zoological, to determine what names should be used to designate diseases, but at the present moment medical terminology is subjective.

In 1843 Dubini, of Milan, Italy, described a hookworm as parasitic in man. Besides the caudal "hooks" (i. e., the "rays") of the male, Dubini's parasite presented four hooks in the mouth. It presented further an anatomical character which is common to all hookworms, namely, the ventral surface of the anterior end grows more rapidly than the dorsal surface, so that the oral end is bent backward like a "hook," and the mouth thus occupies a dorsal position. Thus it is seen that the original character which led to the vernacular name "hookworm" was a misinterpretation; the second character of "hooks" (namely, in the mouth), which has been popularly but erroneously interpreted as responsible for the vernacular name "hookworm," is not present in all species; the hooklike curvature of the head is usually but not always distinct. I propose, however, to retain the word "hookworm" as a vernacular name.

It is not apparent that Dubini knew that Fœrelich had proposed the genus *Uncinaria*, and it is probably on this account that he proposed a new genus—*Agchylostoma* by name—to contain the parasite (*Agchylostoma duodenale*) which he had found in man.

For years it was supposed that this was the only species of hookworm found in man, but in May, 1902, I showed that in America we have a distinct species, which I named *Uncinaria americana*.

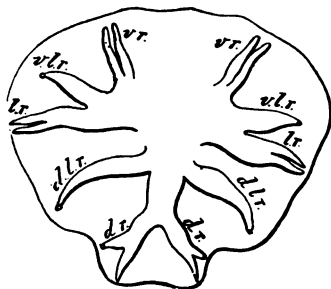


FIG. 1.—Caudal bursa of a male strongyle (*Esophagostoma dentatum*), to serve as diagram for the family: v. r., ventral rays; v. l. r., ventro-lateral rays; l. r., lateral rays; d. l. r., dorso-lateral rays; d. r., dorsal rays. X 93. (After Schneider, 1866, p. 130.)

ZOOLOGICAL POSITION OF THE PARASITES.

The parasites which cause uncinariasis are worms belonging to the nematode family Strongylidæ.

Family STRONGYLIDÆ.

FAMILY DIAGNOSIS.—Nematoda: With body elongate, cylindrical, rarely filiform. Mouth is probably always provided with six papillæ, of which the four submedian are generally salient in form of nodules or conical points. In some cases the mouth is in the axis of the body; in others it is turned dorsally or ventrally, and occasionally provided with a chitinous armature. Esophagus more or less swollen in posterior portion, but without forming in adults a distinct esophageal bulb. Male provided with a caudal bursa, open or closed, entire or divided, and with one or two spicules. Female with one or two ovaries; vulva anterior or posterior of equatorial plane, in some cases near the anus. Eggs deposited during segmentation, in some cases containing embryo.

TYPE GENUS.—*Strongylus* O. F. Mueller.

The sexes are separate and the digestive tract is complete. Characteristic for the family is the presence, on the tail of the male, of an umbrella-like structure known as the caudal or "copulatory bursa,"

supported by a number of finger-like "rays," which may be compared to the ribs of an umbrella. In coitu, the male clasps the body of the female by means of this bursa.

This family is divided into subfamilies, according to the presence of certain anatomical characters. The parasites of uncinariasis are now classified in the subfamily Strongylinæ,^a which, as its former name, Sclerostominæ, indicated, is characterized by the presence of a hard chitinous "buccal capsule."

Subfamily STRONGYLINÆ.

SUBFAMILY DIAGNOSIS.—Strongylidæ: Meromyaria; mouth with more or less complete chitinous armature. Male with two equal spicules; caudal bursa with rays, the dorsomedian and dorsolateral being united in a common base. Female with two ovaries, except in *Ollulanus*.

TYPE GENUS.—*Strongylus* Mueller.

The subfamily Strongylinæ is in turn divided into a number of genera, of which we may mention here the following:

Strongylus [*Sclerostoma*]; the sclerostomes, including the colic worms of horses and the kidney worms of hogs (but not the kidney worms of dogs and man);

Syngamus, including the gape worms of chickens; and

Uncinaria, the hookworms, including the parasite of uncinariasis.

It seems very probable that hookworms will have to be divided into several different genera, for which a new subfamily will perhaps be recognized, but it is not quite clear at present just what genera will be admitted. Undoubtedly *Uncinaria* Frœlich, 1789, must be adopted for one, and in this will probably be placed worms like *Uncinaria stenocephala*, possessing ventral lips but not ventral recurved teeth. It is quite possible that a second genus (*Monodontus* Molin, 1861, or *Bunostomum* Railliet, 1900) will be recognized for certain other forms, with buccal lips and with the prominent dorsomedian buccal tooth, as was proposed by Molin; probably the new American hookworm will be placed in this genus. Hookworms with the ventral recurved buccal teeth, as seen in *Uncinaria duodenalis* and *Uncinaria canina* will probably be separated into a distinct genus, for which Dubini's name *Agchylostoma* will be available. To satisfactorily determine the points at issue will require further anatomical study of a number of different species. For the purpose of this paper it will be sufficient to call attention to these probable changes.

^aFrom a study of the history of the nematode genera, it is very clear that there will have to be a general revision of the technical names of this group. The original *Strongylus*, for instance, was a sclerostome, hence the names *Sclerostoma* and *Sclerostominæ* will have to fall into synonymy. It is probable that *Metastrongylus* will be the correct name for the lung strongyles. *Strongylus contortus* becomes *Hemonchus contortus*. As soon as certain remaining points of this nature are decided, Hassall and I will issue a list of nematode genera, together with their type species.

Genus UNCINARIA^a Frœlich, 1789.

GENERIC DIAGNOSIS.—Strongylinae: With anterior extremity curved dorsally; mouth round to oval, opening oblique, limited by a transparent border and followed by a chitinous buccal capsule; the dorsal portion of the capsule is shorter than the ventral, and is supported by a conical structure, the point of which sometimes extends into the cavity; at the base of the buccal capsule are found two ventral teeth; toward the inner free border the ventral wall bears on each side of the median line chitinous structures, lips (*Uncinaria*) or teeth, often recurved in shape of hooks (*Agchylostoma*); the inner dorsal wall may also bear lips or teeth. Oviparous, eggs with thin, transparent shell.

TYPE SPECIES.—*Uncinaria vulpis*^b Frœlich, 1789.

^aSYNONYMY, WITH ORIGINAL PLACE OF PUBLICATION.

- 1789: *Uncinaria* FRÖELICH<Der Naturforscher, Halle, v. 24, pp. 130–139; type, *Uncinaria vulpis* Frœlich, 1789.
- 1799: *Unciaria* FISCHER<Arch. f. d. Physiol., Halle, v. 3, p. 99. [Apparently a misprint for *Uncinaria*.]
- 1843: *Agchylostoma* DUBINI<Annal. univers. di medic., Milano, v. 106, aprile, pp. 5–13; type, *Agchylostoma duodenale* Dubini, 1843.
- 1845: *Ancylostoma* CREPLIN<Archiv f. Naturg., Berlin, 11. J., v. 1, p. 325; for *Agchylostoma* Dubini, 1843.
- 1845: *Dochmius* DUJARDIN, Histoire naturelle d. helminthes, pp. 267, 275–279; type, *Uncinaria vulpis* Frœlich, 1789.
- 1845: *Docmius* DUJARDIN, ibidem, p. 114. [Misprint for *Dochmius*.]
- (1846): *Anchylostoma* DELLE CHIAJE<Rendicon. dell'Accad. delle Sci. Napoli, v. 5, p. 339. [Not verified.]
- 1850: *Anchylostoma* DUBINI, Entozoografia umana, pp. 102–112; for *Agchylostoma* Dubini, 1843.
- 1851: *Ancylostomum* DIESING, Systema helminthum, v. 2, p. 82; for *Agchylostoma* Dubini, 1843.
- 1851: *Anchylostomum* DIESING, Systema helminthum, v. 2, pp. 321–322; for *Agchylostoma* Dubini, 1843.
- 1861: *Monodontus* MOLIN (not *Monodonta* Lamarck, 1799), Il sottordine degli acrofalli <Mem. r. Ist. ven. di sc., lett. ed arti, Venezia, v. 9, pp. 435, 463–470; type, *M. semicircularis* Molin, 1861.
- 1861: *Dochmius* MOLIN<Ibidem, p. 471. [Misprint for *Dochmius*.]
- 1862: *Dachmius*<Veterinarian, Lond. (416), v. 35, 4. s. (92), v. 8, Aug., pp. 549–556. [Misprint for *Dochmius*.]
- 1879: *Anchilostoma* BOZZOLO<Osservatore, Torino, v. 15 (24), 17 giugno, pp. 369–370; for *Agchylostoma* Dubini, 1843.
- 1895: *Ankylostomum* STOSSICH<Boll. Soc. Adriatica di sc. nat. in Trieste, v. 16, pp. 21–25; for *Agchylostoma* Dubini, 1843.
- 18—?: “*Ankylostoma* Dubini” of various authors; for *Agchylostoma* Dubini, 1843.
- 1897: *Anchylostamum* MEHLAU<Buffalo M. J., v. 36 (8), Mar., p. 573. [Misprint for *Anchylostomum*.]
- 1902: *Dohmius* LOOSS<Contrabl. f. Bakteriolog., Parasitenk. [etc.], Jena,*1 Abt., v. 31 (9), 5. Apr., Originale, p. 424. [Misprint for *Dochmius*.]
- 1902: *Uncinaria* VON LINSTROW<Zool. Centralbl., Leipz., v. 9 (24–25), 16. Dec., p. 778. [Misprint for *Uncinaria*.]

^bThis species is probably identical with *Uncinaria melis* Frœlich, 1789; *Ascaris criniformis* Goëze, 1782, and with *Uncinaria stenocephala* (Railliet, 1884).

The anatomical character which distinguishes the genus *Uncinaria* is the dorsal curvature of the anterior extremity of the body, due to the shortness of the dorsal wall of the buccal capsule and resulting in bringing the mouth into a dorsal instead of a terminal or a ventral position.

In many medical writings this genus is named *Anchylostoma*, a word which is spelled in at least nine different ways, and the disease is spoken of as *anchylostomiasis*. This nomenclature and terminology are due to the fact that when the hookworm (*Uncinaria duodenalis*) of man was first described, in 1843, it was supposed to represent a new genus (*Agchylostoma*). As a matter of fact, however, it is generally acknowledged to be congeneric with a worm described in 1789 as *Uncinaria*. By the international "law of priority," therefore, the names *Agchylostoma*, *Anchylostoma*, etc., fall into synonymy until it can be shown that the two species are not congeneric. (See p. 14.)

In explanation to physicians it may be here stated that zoologists are obliged to deal with hundreds of thousands of technical names, and on this account they have been forced to adopt very rigid rules governing their use. Our most important rule is the "law of priority," which to us is as essential as is the "code of ethics" to the physician.

The genus *Uncinaria* contains blood-sucking worms of the worst type. They are usually not over an inch in length nor thicker than an ordinary hatpin. They are provided with a heavy armature of sharp teeth, by means of which they pierce the intestinal mucosa of their host. They have also an unusually strong muscular esophagus, which serves as a pump during the act of sucking blood. An important point, from the medical aspect of the parasites, is that they do not remain fastened to one spot in the bowels, but suck first at one spot and then at another. Thus the patient loses blood directly to the parasites, and also, by numerous minute hemorrhages, into the intestine. It is probably this latter factor which occasionally gives to the stools of patients that peculiar reddish-brown tinge, and also their occasional bloody appearance.

The injury to the intestinal wall does not stop with the bite. The wound forms an excellent point of attack for bacteria, and the intestinal wall becomes thickened, thus losing, to a greater or lesser degree, the ability properly to perform its function. Not only does the patient lose blood, but his power of assimilation is impaired, and the supply of blood-forming material is thus in part cut off. Some authors also claim that the parasites produce a poison which acts upon the system, a view which is very strongly supported by certain clinical facts.

It was stated above that hookworms are found in various animals. Now, the general rule may be laid down that where these worms are present trouble may be expected.

Uncinaria americana and *Agchylostoma duodenale* cause in man the disease variously known as uncinariasis, uncinariosis, anchylostomiasis, tunnel disease, miners' anemia, brickmakers' anemia, mountain anemia, etc.

Agchylostoma caninum [*Uncinaria canina*^a] causes a similar disease in dogs, resulting, in some parts of the country, in a death rate of from 25 to 40 per cent of the pups born. Uncinariasis in dogs is exceedingly common in Washington, D. C. "Typhoid" in cats is attributed to this parasite.

Instructors in medical colleges who wish to demonstrate hookworms and their eggs to the students will find *A. caninum* of dogs an excellent substitute for *Agchylostoma duodenale* of man in case the latter species can not be obtained.

Uncinaria stenocephala occurs in dogs, foxes, and allied animals, and is causing considerable trouble in the blue fox (*Vulpes lagopus*) industry.

Uncinaria trigonocephala^b is found in sheep and produces a serious anemia. This parasite has been met in Victoria and Calhoun counties, Tex., where, in conjunction with the twisted wireworm (*Hæmonchus contortus*^c), it has caused the death of from 25 to 50 per cent of certain flocks.

Uncinaria radiata^d is found in cattle, producing trouble just below the stomach. The writer has collected this parasite in Dewitt, Gonzales, Victoria, and Calhoun counties, Texas, and has seen specimens from Florida collected by Dr. C. F. Dawson.

Uncinaria Lucasi was found several years ago in the seal pups of Alaska by Mr. Lucas, after whom the worm has been named. It is responsible for about 17 per cent of the deaths of the pups.

Still other species of hookworms are reported for other animals.

None of the species from animals mentioned above is known to affect man, nor has either *Uncinaria americana* or *Agchylostoma duodenale* of man been satisfactorily demonstrated to occur normally in other hosts than man.

^aThis is *Uncinaria canina* (Ercolani 1859) Railliet, 1900, a parasite of canines and felines, which is usually known as *Uncinaria trigonocephala* (Rudolphi, 1809) Railliet, 1885 [not *Uncinaria trigonocephala* (Rudolphi, 1809) Railliet, 1900]. If *Uncinaria* and *Agchylostoma* are recognized as distinct, *U. canina* should be placed in the same genus as *U. duodenalis*. I have not yet tested the correctness of the specific name *canina* for this form, but it is here accepted on authority of Railliet.

^bThis is *Uncinaria trigonocephala* (Rudolphi, 1809) Railliet, 1900 [not "*U. trigonocephala* Rudolphi, 1809"] Railliet, 1885]. Both Railliet and I have recently examined Rudolphi's original material, and it is unquestionably identical with *U. cernua* (Creplin, 1829) of sheep. This species is closely related to *U. americana*.

^c*Strongylus contortus* Rudolphi.

^d*Bunostomum phlebotomum* Railliet.

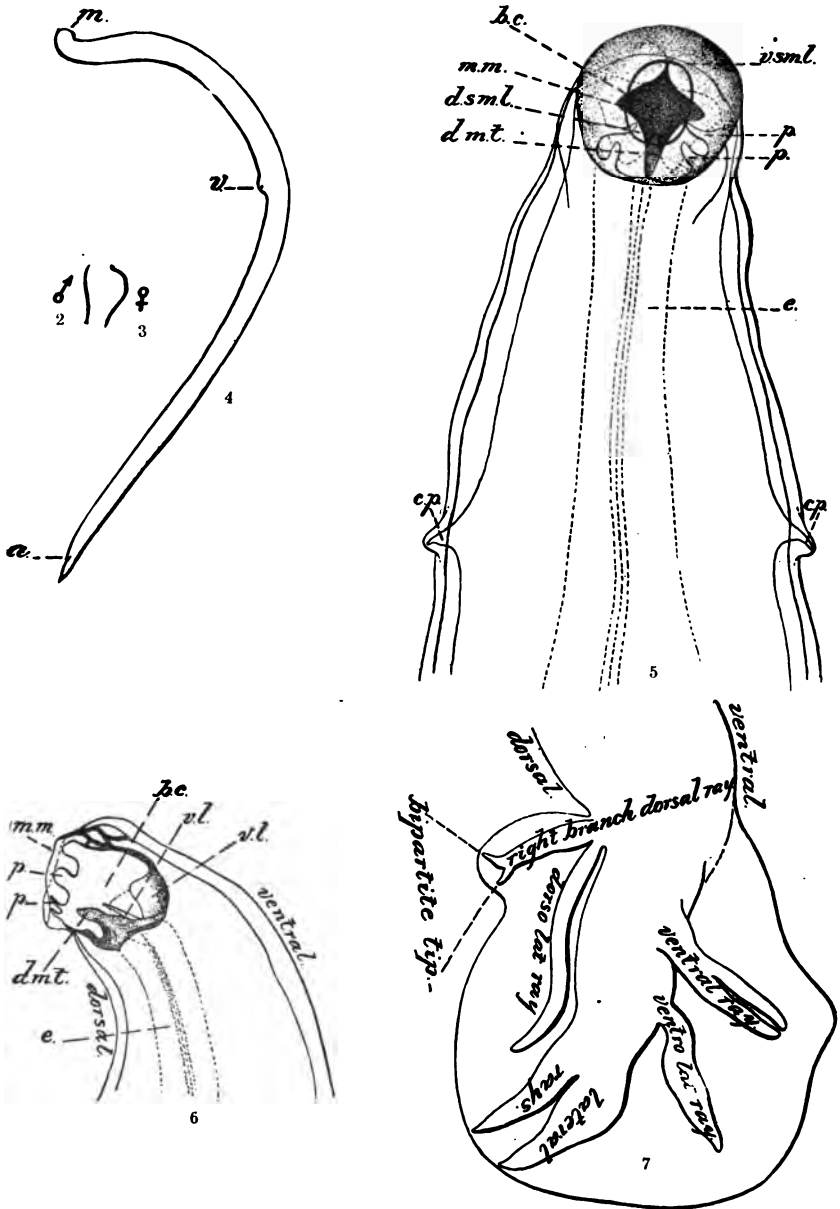


FIG. 2.—New World male hookworm (*Uncinaria americana*). Natural size. (After Stiles, 1902b, p. 190, fig. 120.)

FIG. 3.—New World female hookworm (*Uncinaria americana*). Natural size. (After Stiles, 1902b, p. 190, fig. 121.)

FIG. 4.—The same, enlarged to show the position of the anus (a) and the vulva (v). After Stiles, 1902b, p. 190, fig. 122.)

FIG. 5.—Dorsal view of anterior end of New World hookworm (*Uncinaria americana*): b. c., buccal cavity; c. p., cervical papillae; d. m. t., dorsal median tooth, projecting prominently into the buccal cavity; d. s. m. l., small dorsal semilunar lip; e., esophagus; m. m., margin of mouth, the prominent oval opening seen upon high focus; p. p., papillae; v. s. m. l., large ventral semilunar lips homologous with the ventral hooks of *A. duodenale*. Greatly enlarged. (After Stiles, 1902b, p. 190, fig. 123.)

FIG. 6.—Lateral view of anterior end of New World hookworm (*Uncinaria americana*): b. c., buccal cavity; d. m. t., dorsal median tooth, projecting prominently into buccal cavity; e., esophagus; m. m., margin of mouth; p. p., papillae; v. l., ventral lancets at the base of the buccal cavity, the left lancet is seen en face, the right lancet is seen from the side. Greatly enlarged. (After Stiles, 1902b, p. 190, fig. 124.)

FIG. 7.—Lateral view of caudal bursa of the New World male hookworm (*Uncinaria americana*), showing the arrangement of the rays. Note the short dorsal lobe. Greatly enlarged. (After Stiles, 1902b, p. 190, fig. 125.)

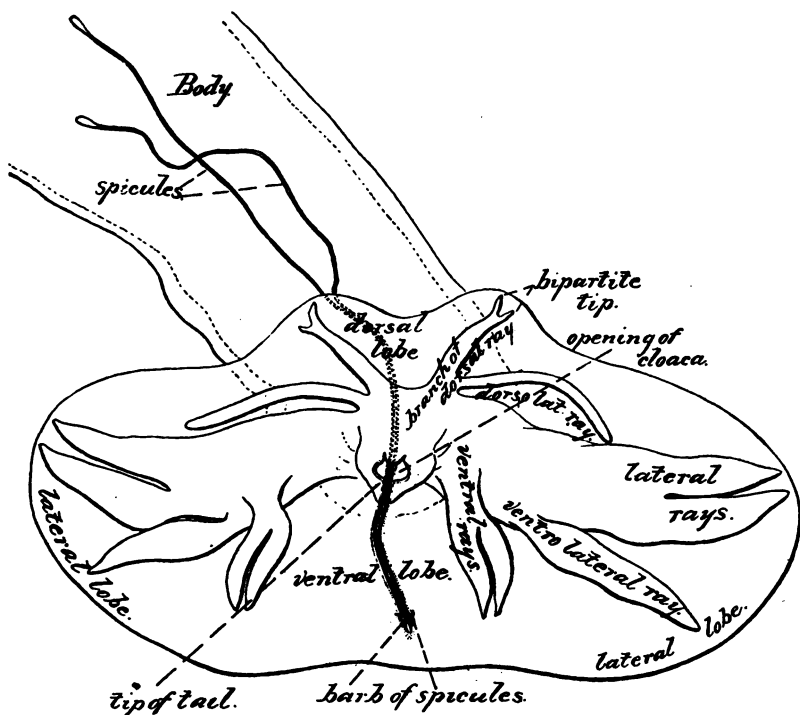


FIG. 8.—The caudal end of the New World male hookworm (*Uncinaria americana*). The bursa is spread out to show the arrangement of the rays. Note the short dorsal lobe which is subdivided, forming two lobes; note also the indistinct ventral lobe connecting the two lateral lobes. The dorsal lobe is thrown back over the body. Greatly enlarged. (After Stiles, 1902b, p. 191, fig. 126.)

[*UNCINARIA* Frølich, 1789, *sensu stricto*].

DIAGNOSIS.—*Uncinaria* s. l. with buccal lips.

TYPE SPECIES.—*Uncinaria vulpis* Frølich.

The New World hookworm—*UNCINARIA AMERICANA*^a Stiles, 1902—of man.

(Figures 2 to 9.)

SPECIFIC DIAGNOSIS.—*Uncinaria*: Body cylindrical, somewhat attenuated anteriorly. Buccal capsule with a dorsal pair of prominent semilunar plates or lips, similar to *U. stenocephala*, and a ventral pair of slightly developed lips of the same nature; dorsal conical median tooth projects prominently into the buccal cavity, similar to *Monodontus*. Male, 7 to 9 mm. long; caudal bursa with short dorso-median lobe, which often appears as if it were divided into two lobes, and with prominent lateral lobes united ventrally by an indistinct ventral lobe; for rays, see figures 7-8; common base of dorsal and dorso-lateral rays very short; dorsal ray divided to its base, its two branches being prominently divergent and their tips being bipartite; spicules long and slender. Female, 9 to 11 mm. long; vulva in anterior half of body, but near equator. Eggs, ellipsoid, 64 to 76 μ long by 36 to 40 μ broad, in some cases partially segmented in utero, in other (rare) cases containing a fully developed embryo when oviposited.

HABITAT.—Small intestine of man (*Homo sapiens*) in America (determined to date, for Virginia, North and South Carolina, Georgia, Florida, Alabama, Texas, Porto Rico, Cuba, and Brazil).

TYPE SPECIMENS.—No. 3310, B. A. I., U. S. Dept. Agric.

^aSYNONYMY WITH ORIGINAL PLACE OF PUBLICATION.

1902: *Uncinaria americana* STILES <Am. Med., Phila., v. 3 (19), May 10, pp.777-778.

1902: *Uncinaria americana* (Stiles) VON LINSTOW <Zool. Centralbl., Leipz., v. 9 (24-25), 16. Dec., p. 778. [Misprint.]

LIFE HISTORY OF *UNCINARIA AMERICANA*.

The life history of the American hookworm has not yet been determined in detail, but there is no reason for assuming that it will differ radically from that of *Agchylostoma duodenale* (see p. 24.)

In my first description of the worm I stated that the egg in the uterus may occasionally contain an embryo. Since making this observation on the females sent to me by Dr. Allen J. Smith I have examined hundreds of fresh eggs, but have not found any containing developed embryos. The question may therefore legitimately arise whether the females originally examined were not exposed to the air for some time before they were preserved, thus making the development of the eggs possible. If the embryo does develop in the uterus, as indicated by some of Dr. Allen J. Smith's material, such an occurrence is undoubtedly rare.

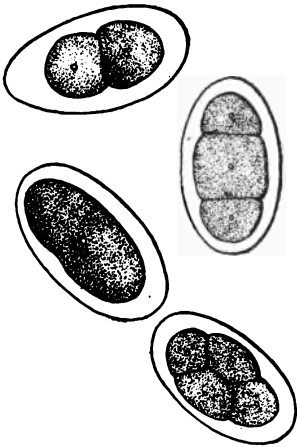


FIG. 9.—Four eggs of the New World hookworm, *Uncinaria americana*, in the 1, 2, and 4 cell stages. The egg showing 3 cells is a lateral view of a 4-cell stage. These eggs are found in the feces of patients and give a positive diagnosis of infection. Greatly enlarged. (After Stiles, 1902b, p. 192, fig. 127.)

DEVELOPMENT OUTSIDE THE BODY.

Segmentation.—In feces exposed to a September, October, or early November temperature of the Carolinas, Georgia, and Florida, the embryo develops in the egg (fig. 9) in about one day's time. In some instances the embryo develops in less than 24 hours. It is a common occurrence to find feces 24 hours old containing free embryos.

The conditions under which the trip was made were not favorable to exact observation in regard to temperature, moisture, etc. It was, however, possible to find worms in their first ecdysis about 2 to 3 days after hatching, and worms in the second ecdysis about 7 to 9 days after hatching. These observations were made under most unfavorable circumstances, when careful measurements, drawings, etc., were excluded, hence they should be repeated.

DEVELOPMENT INSIDE THE BODY.

Experimental infections during the trip were, of course, impossible.

[AGCHYLOSTOMA ^a Dubini, 1843.]

DIAGNOSIS.—*Uncinaria*: Provided with ventral recurved teeth.

TYPE SPECIES.—*Agchylostoma duodenale* Dubini, 1843.

The Old World hookworm—AGCHYLOSTOMA DUODENALE ^b Dubini, 1843, or UNCINARIA DUODENALIS (Dubini) Railliet, 1885—of man.

(Figures 10 to 41.)

SPECIFIC DIAGNOSIS.—*Agchylostoma*: Body cylindrical, somewhat attenuated anteriorly. Buccal cavity with two pairs of ventral teeth curved like hooks, and one pair of dorsal teeth directed forward; dorsal rib not projecting into the cavity. Male, 8 to 11 mm. long; caudal bursa with dorso-median lobe, and prominent lateral lobes united by a ventral lobe; for rays, see fig. 14; dorsal ray divides at a point two-thirds its length from its base, each branch being tridigitate; spicules long and slender. Female, 10 to 18 mm. long; vulva at or near posterior third of body. Eggs, ellipsoid, 52 to 60 μ by 32 μ , laid in segmentation. Development direct without intermediate host.

^aThere are numerous ways of spelling this word (see p. 15), more than one with more or less philological authority. Under these circumstances I adopt the original orthography, despite the fact that it is not philologically correct. By this action I do not intend to necessarily reject the ruling covered by the International Code, but from practical experience I find it impracticable to carry out said rule in reference to the emendation of names until the question of homonyms is decided.

^bSYNONYMY, WITH ORIGINAL PLACE OF PUBLICATION.

1843: *Agchylostoma duodenale* DUBINI <Ann. univer. di med., Milano, v. 106, aprile, pp. 5-13, pl. 1, figs. 1-5; pl. 2, figs. 1-3.

1845: *Ancylostoma duodenale* (Dubini) CREPLIN <Arch. f. Naturg., Berl., 11. J., v. 1, p. 325.

(1846): *Ancylostoma duodenale* (Dubini) DELLE CHIAJE <Rendicon. dell' Accad. delle sci., Napoli, v. 5, p. 339. [Not verified.]

1850: *Ancylostoma duodenale* (Dubini) DUBINI, Entozoografia umana, pp. 103-112.

1851: *Ancylostomum duodenale* (Dubini) DIESING, Systema helminthum, v. 2, p. 322.

?(1851): *Strongylus quadridentatus* SIEBOLD <Naturforsch. Versamml. z. Gotha. [Not verified.]

1861: *Dochmius anchylostomum* MOLIN, Il sottordine degli acrofalli <Mem. r. Ist. ven. di sc., lett. ed arti, Venezia, v. 9, pp. 485-487.

1864: *Sclerostoma duodenale* (Dubini) COBBOLD, Entozoa, pp. 361-362, fig. 77.

1866: *Strongylus duodenalis* (Dubini) SCHNEIDER, Monographie der Nematoden, Berl., pp. 139-140, 1 fig., pl. 9, fig. 3.

1866: "*Ancylostomum duodenale* Dubini" of WHITE <Boston M. & S. J., v. 75 (21), Dec. 20, p. 427. [Misprint for *duodenale*.]

1876: *Dochmius duodenalis* (Dubini) LEUCKART, Die menschlichen Parasiten, v. 2 (3), pp. 410-460, figs. 235-239, 241-247, 249.

1879: *Anchilostoma duodenale* (Dubini) BOZZOLO <Osservatore, Torino, v. 15 (24), 17 giugno, pp. 369-370.

1881: *Dochmius duodenalis* <Rev. méd. de la Suisse Rom., Genève, v. 1 (3), 15 mars, p. 190.

1885: *Uncinaria duodenalis* (Dubini) RAILLIET, Éléments de zool. méd. et agric., Par., pp. 357-359, figs. 245-248.

1897: *Ancylostomum duodenale* (Dubini) MÖHLAU <Buffalo M. J., v. 36 (8), Mar., pp. 573-579. [Misprint for *Ancylostomum duodenale*.]

Ankylostoma duodenale and *Ankylostomum duodenale* of various authors.

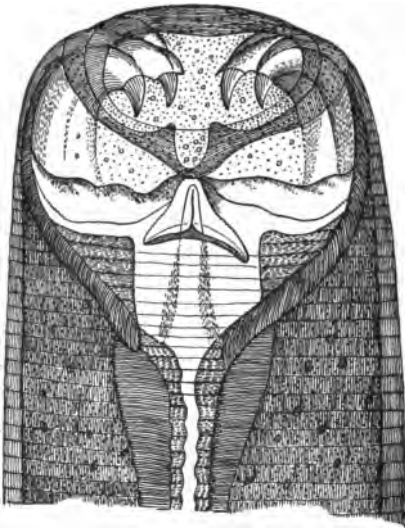


FIG. 10.—Dorsal view of anterior end of the Old World hookworm (*Agchylostoma duodenale*) of man. Greatly enlarged. (After Perroncito, 1882, p. 339, fig. 140.)



FIGS. 11-12.—Old World male and female hookworms (*Agchylostoma duodenale*) of man. Natural size. (After Stiles, 1902b, p. 187, figs. 115, 116.)

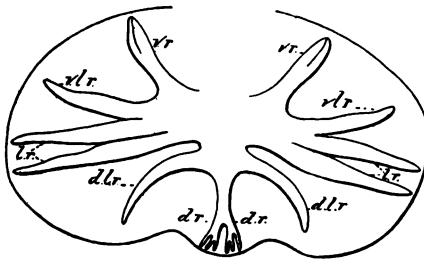


FIG. 14.—Semidiagrammatic figure of the caudal bursa of an Old World male hookworm (*Agchylostoma duodenale*) of man. (After Railliet, 1886, p. 357, fig. 247.)

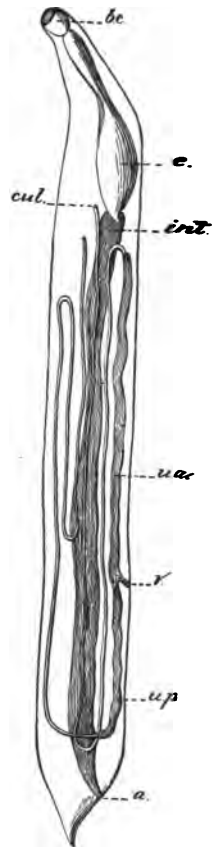


FIG. 13.—Old World female hookworm (*Agchylostoma duodenale*) of man, greatly enlarged diagram to show the anatomy: a., anus; b. e., buccal capsule; cul., cul de sac of ovary; e., esophagus; int., intestine; u. a., anterior uterus; u. p., posterior uterus; v., vulva and vagina. (After Schulthess [copied from Blanchard, 1883a, p. 761, fig. 24].)

HABITAT.—In small intestine of man (*Homo sapiens*); also alluded to in certain apes. Africa, Europe, Asia, Philippines, introduced into America.

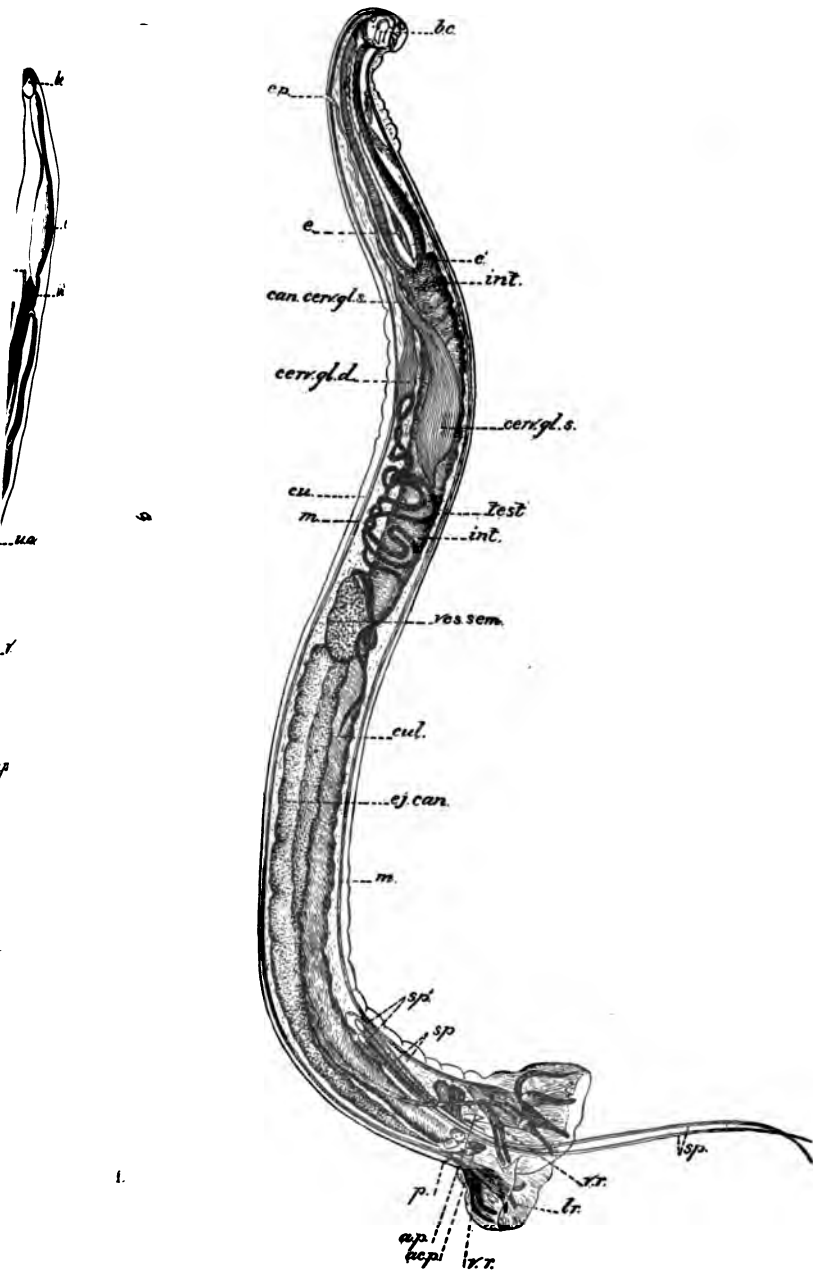


FIG. 15.—Male hookworm (*Agchylostoma duodenale*) of man; ac. p., accessory piece to spicules; a. p., "anal papilla;" b. c., buccal capsule; can. cerv. gl. s., canal of left cervical gland; cerv. gl. d., right cervical gland; cerv. gl. s., left cervical gland; cu., cuticle, cul., cul de sac of testicular tube; e., esophagus; e', posterior end of esophagus; e. p., ventromedian excretory pore; ej. can., ejaculatory canal; int., intestine; l. r., lateral ray of bursa; m., muscular layer; p., lateral pre-caudal papilla; sp., spicules; sp', anterior end of endopod of bursa; test., testis; ves. sem., vesicula seminalis; v. r., ventral ray of bursa. Greatly enlarged. (After Schulthess [copied from 1888a, p. 755, fig. 370].)

LIFE HISTORY OF AGCHYLOSTOMA DUODENALE OR UNCINARIA DUODENALIS.

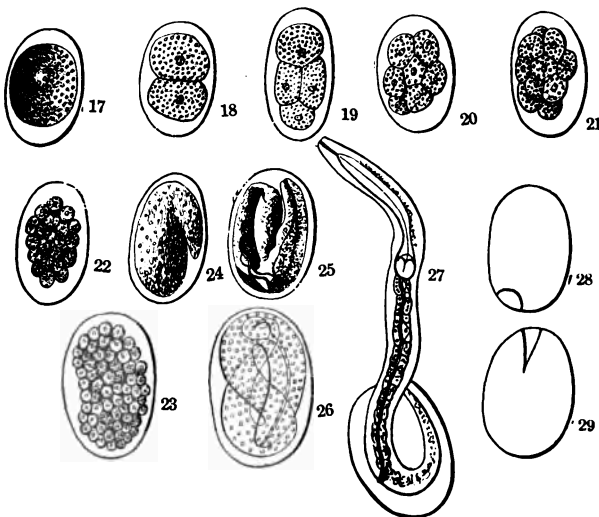
The eggs (fig. 16) are laid in the intestinal tract of the patient by the female worms and are discharged in the feces, either unsegmented or during the early stages of segmentation. They will not develop into adult worms in the intestine, but must first pass out of the body. Thus, for every adult hookworm present in the bowels a separate germ must enter the body.

The egg has a thin shell, which is an indication of a simple life cycle. A short time after escaping in the feces—the time varying according to temperature, moisture, and position in the feces—each egg develops (figs. 17–27) a minute embryo, which is known as a rhabditiform embryo (fig. 27). This name is given to it because of its resemblance to

worms of the genus *Rhabditis*. Characteristic for this stage is the rhabditiform esophagus, which is entirely different from the esophagus



FIG. 16.—Eggs of Old World hookworms (*Agchylostoma duodenale*) as found in the stools. Greatly enlarged. (After Stiles, 1902b, p. 193, fig. 128.)



FIGS 17-29.—Embryology of the Old World hookworm (*Agchylostoma duodenale*) of man; 17-23, segmentation of the egg, 24-26, the embryo; 27, a rhabditiform embryo escaping from its eggshell; 28-29, empty eggshells. Greatly enlarged. (After Perroncito, 1882, p. 342, fig. 142.)

of the adult hookworm. This embryonal esophagus is more or less bottle shaped, and consists of three parts—an anterior elongated

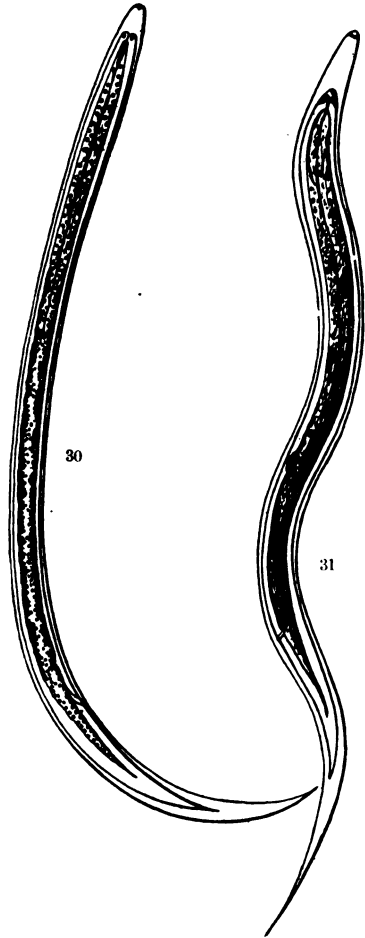
swollen portion, followed by a thin middle portion, the latter being followed by a more or less globular esophageal bulb which possesses a triradiate chitinous armature. This kind of esophagus is common to the early stage of all members of the family Strongylidæ and also to numerous other free-living or parasitic nematodes. It is evidently a worm with an esophagus of this sort which was recently found in the earth taken from the New York tunnel excavations, and upon which was based the report that uncinariasis was present.

The embryo of the hookworm lives in water or moist ground. In its evolution the worm casts its skin four times, thus passing through five stages, and changes its structure so as to assume more and more the characters of the adult. During these changes the sexes become differentiated. Some of these changes occur in water or moist ground, and the rest after infection takes place.

DEVELOPMENT OUTSIDE THE BODY.

Segmentation.—The eggs develop best in the unaltered fecal matter, especially when this is well formed; not so well when it is more fluid in character. The addition of water retards the development, and if considerable water is added the eggs perish. Air is necessary to development, and the eggs nearer the surface of the feces segment more rapidly than those in the center. At a temperature of about 27° C. the embryo may form and escape from the shell in twenty-four hours. Lower temperatures retard development, so that at 21° or 22° C. the embryo may not escape for from thirty-six to forty hours; 1° C. kills the eggs in twenty-four to forty-eight hours, so that freezing weather may be looked upon as disinfecting areas exposed to the cold.

Embryo.—Upon escaping from the shell, the embryo (fig. 27) meas-



FIGS. 30-31.—Two larvæ of the Old World hookworm at the end of the second stage ("encysted larvæ"), representing the young worms retracted from their skin. (After Perroncito, 1882, p. 350, figs. 148 a-b.)

ures 0.3 mm. in length; the anterior end is blunt, the tail long and pointed; 6 points are visible around the mouth, and these develop later into the papillæ; the buccal cavity is $10\ \mu$ long, $1.4\ \mu$ in diameter, and possesses a highly refractive chitinous membrane; the anus is $50\ \mu$ from the tip of the tail; excretory pore $50\ \mu$ from anterior end; $160\ \mu$ from anterior end is seen the primordium of the genital system.

In this stage the embryo takes food and grows. About the second

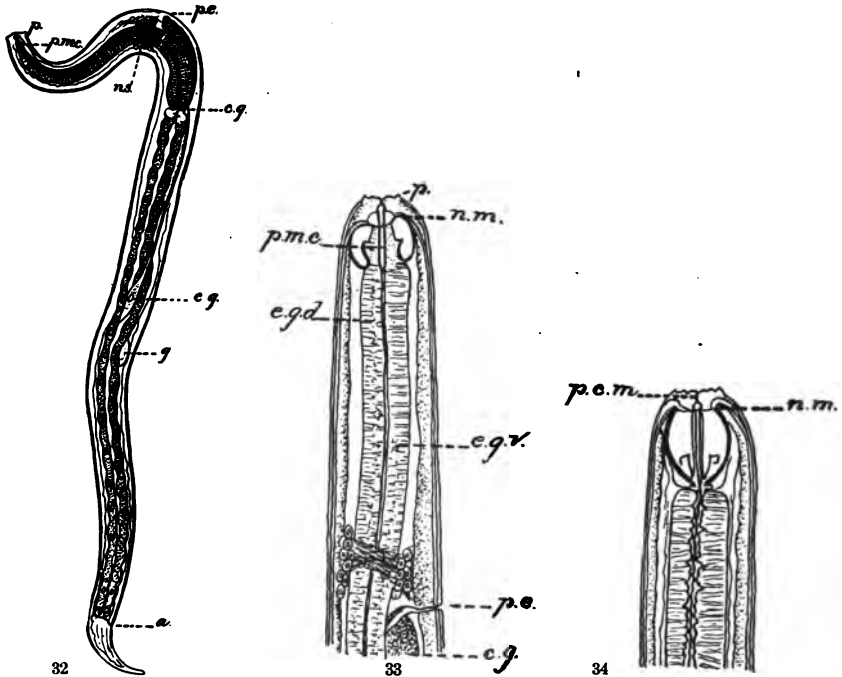


FIG. 32.—A young hookworm (*Agchylostoma duodenale*) of man, without buccal capsule, four days after infection; a, anus; c. g., cervical gland; g, primordium of genital organs; n. s., nervous system; p., papillæ on head; p. e., excretory pore; p. m. c., primary mouth cavity. X about 190 times. (After Looss, 1897, p. 919, fig. 1.)

FIG. 33.—Anterior end of a young hookworm (*Agchylostoma duodenale*) during formation of provisional buccal capsule: c. g., cervical gland; e. g. d., dorsal esophageal gland; e. g. v., ventral esophageal gland; n. m., new mouth; p., papillæ on head; p. e., excretory pore; p. m. c., primary mouth cavity. X 578. (After Looss, 1897, p. 920, fig. 2.)

FIG. 34.—Head of larval hookworm (*Agchylostoma duodenale*) before entering fourth stage, five or six days after infection; n. m., new mouth; p. c. m., primary mouth cavity, which extends through the provisional buccal capsule and continues as lumen of the esophagus. X 578. (After Looss, 1897, p. 921, fig. 3.)

or third day the embryo casts its first skin, but does not change its organization. After about four or five days (at 27°C.) it measures $480\ \mu$ long by $30\ \mu$ in diameter.

Second stage.—After the fifth day the young worms begin to show signs of a second ecdysis, at the same time undergoing certain other changes. Three minute lips, each with two very delicate papillæ, appear under the skin at the anterior end; the brightly refringent cutic-

ular lining of the buccal cavity and the chitinous teeth of the esophageal bulb disappear; the esophagus elongates, becomes thinner, and its three divisions become less distinct; the tail becomes slightly shorter and more blunt; the anus lies $90\ \mu$ from the tip of the tail. The organ-

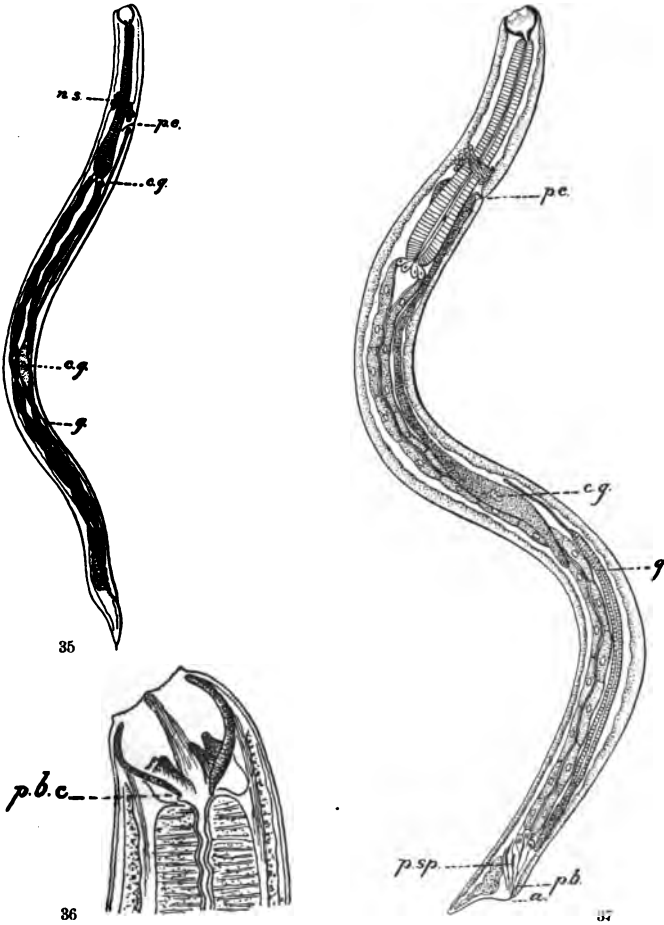


FIG. 35.—Young hookworm (*Agchylostoma duodenale*) in fourth stage, with provisional buccal capsule: c. g., cervical gland; g., primordium of genital organs; n. s., nervous system; p. e., excretory pore. X 105. (After Looss, 1897, p. 921, fig. 4.)

FIG. 36.—Provisional buccal capsule (fourth stage) of a larval hookworm (*Agchylostoma duodenale*), about nine days after infection: p. b. c., primordium of definite buccal capsule. X about 420. (After Looss, 1897, p. 921, fig. 5.)

FIG. 37.—Young male hookworm (*Agchylostoma duodenale*), nine days after infection: a., anus; c. g., cervical gland; g., genital tract; p. b., primordium of bursa; p. e., excretory canal; p. sp., primordium of spicules. X about 105. (After Looss, 1897, p. 922, fig. 6.)

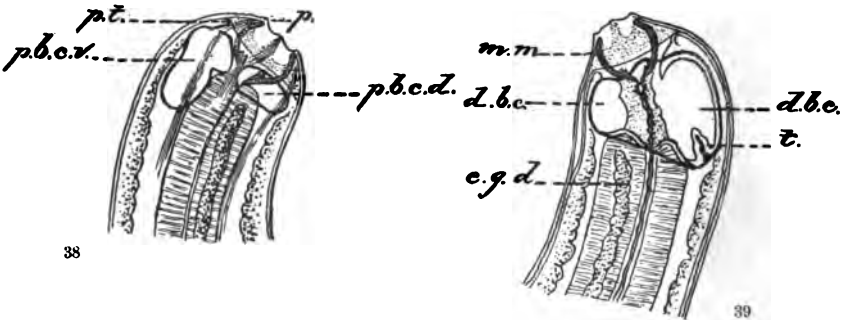
ism becomes more motile, and contracts from its outer skin, thus forming the stage (figs. 30–31) which has been described as an “encystation,” but which in reality is simply a second ecdysis. This is the infecting stage of the hookworm, and ends the development so far as the free life is concerned. No more food is taken. In some cases, however,

the worm escapes from the surrounding cast skin. While water is more or less injurious to the egg and the first stage, the infecting "encysted" stage exists well in this medium, and Looss (1897) succeeded in keeping these worms alive for thirty days in water. Upon drying up the larvæ die, so that the view that the worms exist in dust and are carried around in the air, thus leading to infection, is not well founded (see p. 30).

DEVELOPMENT INSIDE THE BODY.

Upon being swallowed these young worms undergo further ecdyses, changing their internal organization at the same time. We may recognize, with Looss, a third stage, without buccal capsule (fig. 32); a fourth stage, with a provisional buccal capsule (fig. 35); finally, a fifth stage, with the definite buccal capsule, corresponding to the adult form.

Third stage (without buccal capsule, fig. 32).—During their free life the larvæ may attain 0.65 to 0.7 mm. in length by 25 to 27 μ in diam-



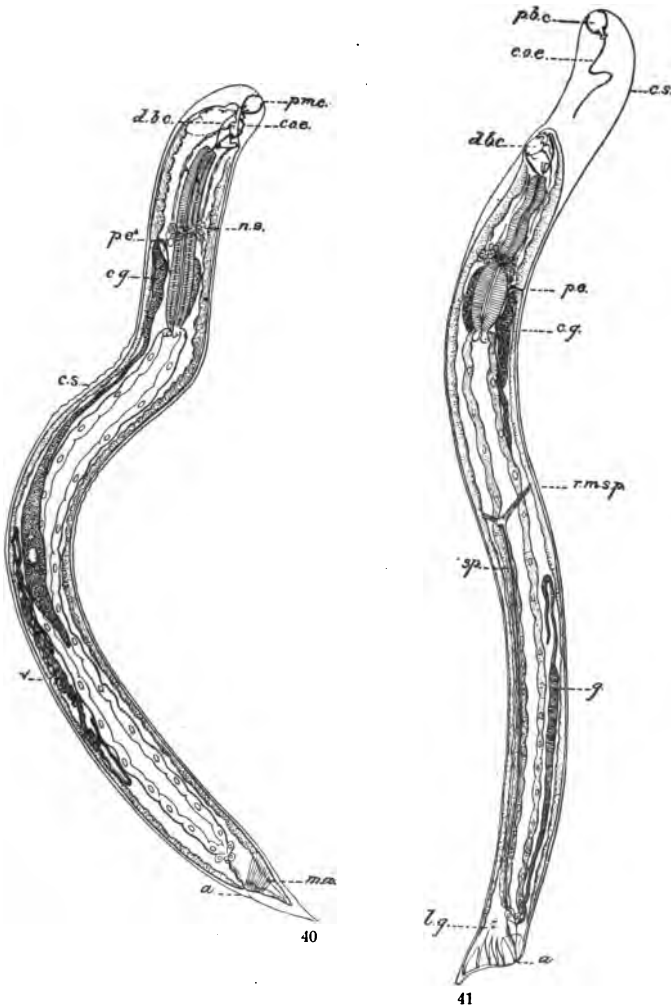
FIGS. 38-39.—Development of definite buccal capsule (38, on twelfth day after infection; 39, one or two days later): *d. b. c.*, definite buccal capsule; *e. g. d.*, dorsal esophageal gland; *m. m.*, margin of definite mouth; *p.*, papillæ on head; *p. b. c. d.*, *p. b. c. v.*, dorsal and ventral primordia of the definite buccal capsule; *p. t.*, primordium of ventral tooth; *t.*, ventral tooth; the new cuticle can be distinguished under the old. X 190. (After Looss, 1897, p. 923, figs. 7-8.)

eter (at the end of the esophagus). The esophagus is 160 μ long, and its three divisions may still be distinguished. The intestine is composed of about 15 rows of two cells each.

Fifteen hours after infection of dogs the worms have passed below the stomach. They now begin to feed, but their growth is comparatively slow. After about five days they begin to show signs of a third ecdysis, which continues until about the seventh day. During this period important changes take place, especially at the anterior end, and result in the formation of the provisional buccal capsule.

Fourth stage (with provisional buccal capsule, fig. 35).—This is the fourth larval stage, namely, the stage after the third ecdysis. The worms have not increased notably in length, but certain organs are advanced in development, and the esophagus no longer shows its former three divisions. The worms measure about 0.66 mm. long by 25 μ in diameter, the latter being nearly uniform for a greater part of the

length (fig. 35). The provisional buccal capsule attains $40\ \mu$ in diameter, and the mouth is bent slightly dorsad. Two pairs of teeth are visible at the base of the capsule—one pair situated dorsally, the other ventrally. During this stage the animal increases in length and thick-



FIGS. 40-41.—Male and female hookworms (*Agchylostoma duodenale*) during the fourth casting of skin 14 to 15 days after infection: a., anus; c. g., cervical gland; c. o. e., cuticle of old esophagus; c. s., cast skin; d. b. c., definite buccal capsule; g., genital organs; l. g., large ganglion, supplying the rays of the bursa; m. a., anal muscle; n. c. gl., nucleus of cervical gland; p. e., excretory pore; p. b. c., provisional buccal capsule; r. m. sp., retractor muscles of spicules; sp., spicules; v., vulva. X 42. (After Looss, 1897, p. 924, figs. 9-10.)

ness, the inner organs become better developed, the sexes become differentiated, and the definite buccal capsule forms at the anterior end. With these changes the parasite prepares for its last—namely, a fourth—ecdysis, which occurs about fourteen to fifteen days after infection.

Fifth stage (with definite buccal capsule).—The worm is now about 1.9 (male) to 2 mm. (female) long, 12 to 14 μ in diameter—very much smaller than the adult forms. It is estimated that the parasites require about four to five or six weeks from the time of infection to become mature.

SOURCE OF INFECTION OF UNICINARIASIS.

The worms may be swallowed in contaminated food or in drinking water during or after the second ecdysis. Persons handling dirt are especially apt to get the microscopic worms on their hands, and it is an easy matter to transfer them to the mouth, either directly by biting the finger nails or sucking the fingers, or indirectly with food. In prevention, therefore, careful personal habits and pure drinking water are indicated.

Some writers state that the young stages are scattered in the air by the wind and in a dry state, the inference being that they may then be breathed in or may contaminate food. While not caring to go to the extreme of stating that such a method of dissemination or infection is impossible, my own observations on this class of parasites do not lead me to attach any importance to aerial infection. As a rule, drying-out results in a high mortality among nematode larvæ, embryos, and eggs, while moisture, on the other hand, is necessary for their existence. Now, uncinariasis is not so common as to fill the air with dried larvæ, and the chances of inhaling the latter appear to be almost infinitesimally small. Since the worms do not increase in number in the intestinal tract, we should therefore expect (in case aerial infection were common) to find rather few cases of severe infection, but a more or less uniform light infection of nearly all persons or animals inhabiting an infected area, since all are breathing the same air. Further, as adults breathe more air than children we should expect the former to present the cases of heaviest infection. We should also expect to find the disease more general in dry years than in wet seasons. Such, however, does not appear to be the case. Cases of infection vary greatly in intensity, and the losses from nematode diseases in sheep are much less in dry years and in dry localities than in wet seasons. As a matter of fact, few factors can be conceived of which would probably result in killing more germs of the disease than would dry winds.

In preventing uncinariasis and nematode diseases in general among live stock, systematic draining and burning of pastures are strongly advocated.

Looss (1898, 1901) recently suggested that the larvæ may enter the human body by way of the skin and then pass through the body to the intestine. Startling as this view is, Looss (see Sandwith, 1902) has recently demonstrated the correctness of it. (See p. 59.)

BRIEF REVIEW OF UNCINARIASIS.

Uncinariasis is by no means a new disease. According to Sandwith (1894), a medical papyrus, written about three thousand four hundred and fifty years ago, embraced in an encyclopedic form the knowledge at that time of Egyptian teachers. This oldest of all books among medical works (Eber's papyrus) came into the hands of Professor Ebers at Thebes, in 1873, and has recently been translated into German. Dr. Joachim (1890) and Scheuthauer (1881) agree that anemia, due to hookworms (*Agchylostoma duodenale*), was well known to physicians of those days under the name of "AAA" and "UHA." The papyrus describes accurately among the symptoms, "heart weakness, palpitation, stabbing cardiac pains, constipation, edema of the legs, a weight in the body pressing heavily, and other digestive troubles." It further prescribes a remedy for a patient who has in his body worms, which are produced by the "AAA" disease, and possibly it is the hookworms which are referred to.

Within modern times this special form of anemia was described in Brazil by Piso in 1648; Labat (1742 or 1748) observed it in Guadeloupe, Chevalier (1752) in St. Domingo, Dazille and Bason (1776) in the Antilles, and Edwards (1790 or 1793) in Jamaica. In Europe, the disease was first noted among the miners of Anzin in 1802.

Not until 1843 was the parasite (*Agchylostoma duodenale*) described, when Dubini of Milan published an account of it. Later it was reported from Egypt, Germany, France, India, Ceylon, Japan, Australia, and elsewhere, and to it was attributed a certain widespread anemia of brickmakers, tunnelers (St. Gothard tunnel anemia), and miners.

Zinn and Jacoby (1898), who have compiled 464 bibliographic references to the disease, give two charts showing its distribution at the time their paper was published.

In studying the maps, it will be well to recall that at the time they were printed nothing was known regarding the relations of uncinariasis to the soil (see p. 47), hence, the areas which are given as infected are probably much greater than the actual extent of the infested territory; further, the maps would indicate that they have registered the places in which hookworm disease has been diagnosed, and not necessarily the areas in which hookworm infection occurs.

In connection with their references to the United States, they simply mention Georgia, Alabama, and Louisiana, saying that there is little to report upon this subject for these localities.

BRIEF REVIEW OF UNCINARIASIS IN THE UNITED STATES.

In order to understand the American publications on this disease, it must be stated that much of the so-called "dirt-eating," "pica," "cachexia africana," antebellum "negro consumption," "mal d'estomac," "malnutrition," and "malarial anemia," described for the Southern Atlantic States, is in reality due to uncinariasis.

The earliest reference which seems quite positively to refer to this disease in this country, so far as I have yet found, is an article by Dr. Pitt (1808, pp. 340-341), who says that along the Roanoke River, North Carolina, malacia or dirt-eating "prevails mostly among the poor white people and negroes, and originates, in my opinion, from a deficiency of nourishment." Chabert's (1821) description of the conditions of the slaves of Louisiana, which he attributed to dirt-eating, Jordan's (1832, of Person County, N. C.) account of "cachexia africana, or negro consumption," Cotting's (1836) account of the dirt-eating in Richmond County, Ga., Little's (1845) description of the dirt-eaters of Florida, Le Conte's (1845) account of the dirt-eating in the pine barrens of Georgia, Duncan's (1850) record of dirt-eaters in St. Mary's Parish, La., all apply so well to the uncinariasis I studied in some of the same States that I have no hesitation in assuming that many, if not all, of the cases were due to infection with *Uncinaria*. Lethermann (Florida), Lyell (Georgia and Alabama), and Heusinger and Geddings (South Carolina) are said to have published on similar conditions in the South, but not being able to trace their articles, I am unfortunately unable to give them full credit for whatever views they may have advanced.

Blickhahn (1893a) seems to have been the first physician to recognize as such and to publish a case of uncinariasis for this country. The patient was a German brickmaker who had been in the United States seventeen months, and Blickhahn believes the infection took place in Germany. It is true that Herff (1894) records a case of supposed uncinariasis observed in Texas in 1864, and Allen J. Smith (published by Schaefer, 1901) found *Uncinaria* eggs in feces of man in Texas in 1893, but these publications are antedated^a by Blickhahn's article; hence Blickhahn has priority of discovery. It is, however, interesting to note that Blickhahn's case, being in a German, was probably caused by the Old World parasite, *Agchylostoma duodenale*, while the cases of Herff and Allen J. Smith were in all probability the first endemic cases recognized. Herff did not appear altogether certain regarding his diagnosis, but his short account of the worm indicates that the interpretation is correct.

^a In all zoologic matters neither priority of observation nor priority of presentation before a scientific society avails to give priority of discovery. By international agreement, and by custom extending back a century and a half, zoologists recognize only actual publication as governing a question of this sort.

Möhlau (1896) reported five cases for Buffalo, N. Y., which were supposed to be due to the Old World parasite (*Agchylostoma duodenale*). Gray (1901) recorded two cases for Richmond, Va., contracted elsewhere in the same State, which he states positively (personal conversation) were due to *Agchylostoma duodenale*, and Tebault (1899) recorded a case of uncinariasis in a boy of German descent in New Orleans.

To one of my former pupils, Dr. Bailey K. Ashford (1900), of the U. S. Army, is unquestionably due the credit of having first seriously directed the attention of American physicians and zoologists to this disease.^a Ashford in fact found this malady very common in Porto Rico, and although he erroneously considered the parasite to be identical with the Old World species, his clinical observations placed the American medical profession on its guard for cases which might occur in returning American troops.

In 1901 there was a sudden increase in American observations. Dyer (March 15, 1901) reported a case for St. Louis. Schaefer (May, 1901) was reported in the proceedings of the Texas Medical Association (Texas Medical News, May, 1901) as presenting a paper on a new form of intestinal parasite in Texas. The case in question was uncinariasis, probably due to *Uncinaria americana*, but was not published as uncinariasis until October 26, 1901.

Claytor's (June, 1901) case is, so far as I am aware, the first published American case which can be recognized as unquestionably due to *Uncinaria americana*, although at least some (and probably most, if not all) of Ashford's cases were caused by this species. Claytor's case was originally published as an infection with the Old World species, *Agchylostoma duodenale* (an error for which the responsibility rests upon me, not upon Dr. Claytor; the large-sized egg found should have placed me more on my guard).

Allyn and Béhrend (July 13, 1901) recorded an imported case in an Italian boy in Philadelphia, due apparently to *Agchylostoma duodenale*, and at the same time mentioned three unpublished cases diagnosed by Dr. L. Napoleon Boston, two in 1900 and one in 1901. All three cases came from Porto Rico, so that they were probably due to *Uncinaria americana*.

^a It is true, as has been stated, that for some years prior to Ashford's publication I had repeatedly insisted upon the probability of the frequent occurrence of this disease in the United States, having discussed the subject in my lectures on medical zoology in the post-graduate medical school of the U. S. Army, and in the medical classes of Johns Hopkins University and of Georgetown University (the latter, the alma mater of Dr. Ashford), as well as in various medical meetings; but so far as I am aware, my views were not printed until July, 1901, so that Ashford's printed statements antedate mine by more than a year. Furthermore, his paper was a practical demonstration, while my views were theoretical deductions.

In a paper (July, 1901) written for the Texas Medical News I expressed very positive views to the effect that uncinariasis in man must be more or less widespread in the United States, and I discussed the disease in general. The position taken was based upon general zoologic principles, and, so far as I am aware, was the first printed definite claim regarding the frequency or probable frequency of the malady in the United States.

Schaefer (October 26, 1901) next published a case for Galveston, Tex., probably infected in Mexico, and made the very important statement that Allen J. Smith had found one case in Galveston in 1893, and since then that he had encountered two [afterwards six additional] cases among some 80-odd medical students of the University of Texas.

The importance of this discovery by Allen J. Smith should not be underestimated. To the clinician it did not mean very much, since no record existed that the students exhibited any very severe symptoms. To the zoologist, however, it meant a practical demonstration that uncinariasis was more or less common in the South. Here were 3 [9] students in a city (Galveston); the chances that the infection took place in Galveston did not seem very great; as the students came from different places (according to personal information), the infection must be more or less widespread; and since light cases occurred among medical students, heavier infections must naturally occur among persons who come more regularly in contact with the dirt. Allen J. Smith's observations, the importance of which has not yet been duly recognized by medical journals or by his colleagues, led to some correspondence between himself and me, and he very kindly forwarded specimens from one of his cases. In some respects these parasites resembled *Uncinaria stenocephala* of the dog, and both Allen J. Smith and I were fully agreed that they were not identical with *Agchylostoma duodenale*. I obtained specimens from Claytor's case and also some material which Ashford had sent from Porto Rico to the U. S. Army Medical Museum. All three lots of worms agreed with each other, and differed from *Uncinaria stenocephala*, which I had obtained from Europe, as well as from *U. trigonocephala*^a from sheep, *U. radiata* from cattle, *U. Lucasi* from the Alaskan seal, and from every other species of *Uncinaria* of which I could obtain either specimens or description. Accordingly, I described (May 10, 1902) these worms as a new species, naming it *Uncinaria americana*.

Having now an endemic species, with specimens in my possession for Washington, D. C. (patient came from Virginia), Porto Rico (Ashford's material), Cuba (specimens sent by Guiteras), and Galveston (Allen J. Smith's material), I did not hesitate to state positively in

^a *U. cernua* (Creplin, 1829). See Stiles, 1902b, p. 189.

a paper published in the Eighteenth Annual Report of the Bureau of Animal Industry, and issued on September 25, 1902, that we must have in the United States an endemic uncinariasis which had been generally overlooked.

Prior to the appearance of the paper in question, Claude Smith, of Atlanta, Ga. (June, 1902), had presented a case of uncinariasis before the American Medical Association (see below, p. 103), the parasite afterwards proving to belong to the species *Uncinaria americana*.

H. F. Harris (July 19, 1902), also published a case of uncinariasis (see below, p. 103) for Georgia. In reply to a letter from me, he stated (August 9) that he had found other cases also, and that he was "absolutely sure this disease is very common in this [Porter Springs] locality."

Upon my transfer from the United States Bureau of Animal Industry to the United States Public Health and Marine-Hospital Service, I decided upon an early study of uncinariasis in man, to determine its frequency and geographic distribution in the Southern States. A preliminary report (Stiles, 1902c, October 24) was published, giving my results for Virginia, North Carolina, and part of South Carolina. This report reads as follows:

[Reports to the Surgeon-General Public Health and Marine-Hospital Service.]

HOOKWORM DISEASE IN THE SOUTH—FREQUENCY OF INFECTION BY THE PARASITE
(*UNCINARIA AMERICANA*) IN RURAL DISTRICTS.

(Preliminary report by Dr. Ch. Wardell Stiles, chief of Division of Zoology, United States Public Health and Marine-Hospital Service, detailed for this investigation.)

KERSHAW, S. C., October 22, 1902.

SIR: Through the director of the Hygienic Laboratory, I have the honor to submit a brief preliminary report in regard to the frequency and geographic distribution of hookworm disease (uncinariasis) in the Southern States.

Meeting the disease in the Virgilina copper-mine district of southern Virginia and northern North Carolina, I have thus far traced it through the coal-mine district of Cummock, N. C., the brickyards of Camden, S. C., and the granite-sand district of Lancaster and Kershaw counties, S. C.

The present indications are that it is more prevalent in sandy regions than in clay or stone districts. On the farms and plantations of the sand region of the two counties just mentioned, it appears to be the most common disease of man, and from an economic point of view it appears to be of great importance. The extreme cases seem to occur more commonly among children and women than among adult males over 25, but the present facts at my disposal do not indicate that the malady is quite so fatal as the European form of the disease caused by *Uncinaria duodenalis*. All of the cases thus far examined are due to *Uncinaria americana*, demonstrating clearly that this is an endemic infection and totally independent of the cases which have been introduced from Europe, Asia, and northern Africa.

In general, it may be said that the "pale skin," the "heart disease," the "diarrhea," the "bloat," and the suppression of menses which I have thus far examined all represented various stages of heavy infections with *Uncinaria americana*, and it is impossible to escape the conclusion that so far as the farms and plantations

are concerned a radical change in the general therapeutics practiced in the localities in question is urgently indicated.

As for the economic side of the problem, it should be recalled that the disease in question is resulting in loss of wages, loss in productiveness of the farms, loss in the school attendance of the children, extra expenses for drugs and for physicians' services, etc.

The heavy and frequent infections found are amply explained by the almost total absence of privies and closets on the farms visited. Defecation occurs at almost any place within a radius of 50 meters from the house or hut, and as a result the premises become heavily infested with the embryos.

The disease as thus far traced is primarily a "poor man's" malady, and in frequency it far exceeds even the most extreme limit which theoretical deductions seemed to justify before commencing the field work. There is, in fact, not the slightest room for doubt that uncinariasis is one of the most important and most common diseases of this part of the South, especially on farms and plantations in sandy districts, and indications are not entirely lacking that much of the trouble popularly attributed to "dirt-eating," "resin-chewing," and even some of the proverbial laziness of the poorer classes of the white population are in reality various manifestations of uncinariasis.

The infection among the miners, so far as discovered, is less severe and less common than the infection on the farms and plantations of the sandy districts.

Respectfully,

CH. WARDELL STILES, Ph. D.,
Chief of Division of Zoology.

On November 15, Dr. H. F. Harris, of Atlanta, Ga., published an important notice regarding uncinariasis in the South. After referring to his first case (see above, p. 35), he says:

"The discovery of a distinct American species of the hookworm is very important, as it leads to the inference that the aborigines of this country were infested with this parasite, and that the worm is probably present in all parts of the United States where the conditions are suitable for its development.

"My observations during the last six months bear out this assumption in a most striking manner. A few weeks after my first case of the disease was seen, a second one was encountered that originated in middle Georgia, but though I was constantly on the search for it no other case was found among the numerous patients that come to the clinics of the Atlanta College of Physicians. In June of the present year I made a trip to north Georgia, a region that has long been noted as one in which the inhabitants are very pale and anæmic, this condition being commonly reputed to be the result of dirt eating. Here I saw many instances of what was in all probability ankylostomiasis; but as a result of the ignorance of the people and their suspicion of all strangers a proper examination could be obtained in only four cases, in all of which the parasite was demonstrated. Subsequently a case of the disease was seen that originated in middle Alabama. During September and October I have been studying malaria in south Georgia and Florida, a region in which the people show profound anæmia even more often than in north Georgia. This condition is commonly ascribed to malaria, but my observations show that in almost all instances the sufferers have no malarial parasites in their blood, but eggs of the ankylostoma are constantly found in the feces. During my entire stay in this region I only saw one case of profound anæmia from malaria, and in this instance the patient did not exhibit the extraordinary anæmia so commonly found in those infected with the ankylostoma. I feel no hesitation in saying that time will show that by far the greater number of cases of anæmia in Georgia, Alabama, and Florida are due not to malaria but to the ankylostoma, and that this is the most common of all the serious diseases in this

region. There can be no reasonable doubt that what is true as regards the States named likewise holds good for the entire South. Since my first case was reported 13 other instances of the disease have been seen—11 originating in this State, and 1 each in Florida and Alabama; and if all of those encountered who were suffering from anæmia could have been examined there can be no doubt that the number would be many fold greater.

"This communication is written in the hope that Southern physicians will take up this most important matter at once, for in no other serious disease does the victim suffer so long, in no other condition is he for such a period a menace to those about him, and in no other malady of such gravity is the treatment so rapidly and surely successful."

ITINERARY OF TRIP THROUGH THE SOUTHERN ATLANTIC STATES.

DISTRICT OF COLUMBIA.

In September, 1902, with the aid of three assistants (Messrs. P. E. Garrison, B. H. Ransom, and E. C. Stevenson) I began a systematic study of animal parasites among the patients of Government Hospital for the Insane, District of Columbia. From September 10 to December 12, 1902, the stools of 500 male^a patients were examined microscopically and 15 patients, or 3 per cent, were found to be infected with hookworms. The history has not yet been examined for each case, but probably most of the patients became infected in Cuba, Porto Rico, or the Philippines.

VIRGINIA.

Richmond and State farm.—Starting on my field work, my first stop (September 25) was Richmond, Va. Through the courtesy and cooperation of Dr. Charles V. Carrington I was able to examine at the State penitentiary and the State farm nearly 1,200 convicts. The prisoners filed past Dr. Carrington and myself in single file, and we selected those who appeared anemic or debilitated. Those selected were sent to the hospital and kept there until specimens of feces were obtained. In microscopic examination of 6 white male convicts no case of uncinariasis was found. The only zooparasitic case noticed was an exceedingly heavy infection with whipworms (*Trichuris trichiura*) in a man about 70 years old.

In 22 male negroes the examination was likewise negative so far as *Uncinaria* was concerned, but one case of infection of eelworms (*Ascaris lumbricoides*) was found. In several of the convicts starch digestion or meat digestion was poor, but in most instances the debilitated condition was due to tuberculosis or other diseases.

At the almshouse in Richmond 1 white female 28 years of age gave negative results; a weak-minded girl of 7 years showed a heavy infection with whip worms (*Trichuris trichiura*). In two negroes (1 male, 28 years; 1 female, 30 years) the examination was negative.

^a In 350 female patients examined to March 1, 1903, only one case of hookworm infection has been found.

It proved to be so difficult to obtain specimens of feces from the brickyards that no microscopic examinations were made. No clew was obtained to any disease among the workers which could be interpreted as probable uncinariasis.

Besides Dr. Carrington, I am indebted to Dr. Staton also for cooperation in my work at Richmond.

NORTH CAROLINA.

Virgilina Copper Mine district, southern Virginia and northern North Carolina.—Proceeding to the Virgilina copper mine district I found it very difficult at first to obtain specimens for examination. At one mine 1 white male and 3 negro males were examined; all were negative except 1 negro 22 years old, who showed infection with *Ascaris lumbricoides*. At this mine defecation under ground is prohibited; it occurs in the surrounding woods, at any place within a radius of about 50 meters from the shaft.

At a second mine three specimens of feces were taken at random from the woods; eggs of *Uncinaria americana* were found in one specimen, and ova of *Ascaris lumbricoides* in a second. The patients could not be traced. Specimens were then obtained from 4 white and 18 negro miners, the feces being taken without reference to the physical condition of the men. Of these 22 men, 1 negro 25 years old showed a light infection with *Uncinaria americana*, and inquiry developed the fact that he "had not been well for some time." His chief complaint had been "diarrhea." In two other cases, a white man 41 years old and a negro 34 years old, eggs of the eelworm (*Ascaris lumbricoides*) were found. At this mine defecation under ground is prohibited, and a box privy is located within about 50 meters of the shaft. The men prefer, however, to defecate in the surrounding woods.

I am indebted to Drs. F. D. Drewry and P. P. Causey, and to Mr. L. N. White, manager of the Person Consolidated Copper and Gold Mines Company at Durgy, N. C., for their kind cooperation in connection with my work in Virgilina and vicinity.

Cumnock Coal Mines, Chatham County.—The miners at this place were so suspicious regarding my work that it was almost impossible to obtain specimens for examination. In fact only two specimens could be obtained from about 40 men. One of these, a white miner, 52 years of age, and in rather anemic condition, showed infection with *Uncinaria americana*. The other, a white engineer, 34 years old, gave negative results.

Sanford, Moore County.—Specimens from 4 whites (2 males, and 2 females), obtained by 2 of the local physicians, were examined with negative results.

SOUTH CAROLINA.

Camden, Camden County.—Unexpectedly delayed at Camden, I visited the brickyards with Dr. J. W. Corbett. Of 7 specimens of feces picked up at random from the ground, probably most if not all from negroes, 1 was found infected with *Uncinaria americana*. One white laborer also showed infection with the same parasite.

Haile Goldmine, Lancaster County.—Through the kindness of Captain Thiess, the superintendent of the mines, and Dr. Gregory, the local physician, I was able to examine specimens from 5 white men and 5 negroes connected with the mine. All of these examinations were negative.

Upon leaving Richmond I happened to recall the observation made by Lucas (in Jordan & Clark, 1898, p. 70) in connection with uncinariasis of the seal pups of Alaska, namely, that the infected animals were almost invariably found on the sand rookeries; I also recalled that I had observed severe outbreaks of uncinariasis in sheep and goats on more or less sandy pastures, and further that a severe outbreak of the same disease among dogs had once been reported to me as occurring in a sandy yard. Not recalling at the time any similar observation for uncinariasis in man, I determined to test the subject at the first opportunity, and from Richmond to Haile Goldmine I had diligently inquired of every physician I met, whether he found more anemia on sand, clay, or rock soil. Most physicians replied that they had paid no attention to the subject; one physician stated that he thought anemia was more common in sandy than in clay localities.

Through Captain Thiess I learned that the land near the mines was chiefly a granite sand. With Dr. Gregory, I drove about 4 miles into the sandy district in Lancaster County and found a family of 11 members, one of whom was an alleged "dirt-eater." The instant I saw these 11 persons I recalled Little's (1845) description of the dirt-eaters of Florida. (See Stiles, 1902b, p. 208.) A physical examination made it probable that we had before us 11 cases of uncinariasis, and a specimen of feces from one of the children gave the positive diagnosis of infection with *Uncinaria americana*. There were hundreds of eggs present.

Inquiring for the largest plantation of this sand district, I was directed to a place in Kershaw County, about 6 miles from Kershaw, and through the kindness of Dr. Twitty and the owner of the plantation, I was able to make the desired examinations.

There are about 60 white "hands" on this farm. Going to a field I found about 20 at work. These 20 persons, men, women, and children, corresponded in more or less detail to the description of the so-called dirt-eaters and resin-chewers. A physical examination

showed that they also corresponded to cases of uncinariasis. A family of 10 members was selected and examined carefully. Specimens of feces from 4 of them were examined microscopically and found to contain hundreds of eggs of *Uncinaria americana*. The owner of the plantation informed me that it would be a waste of my time to examine the remaining 40 "hands," as they were in exactly the same condition as the 20 already examined.

Driving to a neighboring farmhouse, I found a family of 5 members, 3 of whom presented such severe and typical symptoms that I had no hesitation in diagnosing them as due to uncinariasis.

Kershaw County.—While driving back to Kershaw, I passed a country schoolhouse. The children, about twenty-five or thirty in number, were at play during recess, and a mere glance at them was sufficient to show that 30 to 40 per cent presented the same general appearance as the children on the neighboring plantation.

At Kershaw several extreme cases were met on the street. The persons in question had come in from the country. One farmer, living about 9 miles away from Kershaw, had with him two of his children. He stated that his entire family, 10 in number, had suffered or were suffering in the same way as these two boys. Physical examination made uncinariasis probable, and the microscopic examination of the feces showed heavy infections with *Uncinaria americana*.

Inquiry among the local physicians and the more intelligent laity elicited the information that the cases that I had seen represented conditions which were usually attributed to "dirt-eating," "resin-chewing," "heart disease," "bloat," "amenorrhea," "anemia due to malaria" (mosquitoes were noticeable chiefly by their absence), "general debility," "poor nourishment," etc. I was further assured that these conditions were general throughout this region, and were not, or only slightly, amenable to treatment.

Taking these cases together, some forty or fifty in all, which I examined carefully within three days, we have one common symptom, namely, *anemia*; nearly all other symptoms noticed could be reduced to sequelæ of *anemia*; further, in every case examined microscopically, exceedingly heavy infections with *Uncinaria americana* were found. Under these conditions, and because the general clinical history corresponded so well with uncinariasis, I have not the slightest hesitation in grouping the cases observed as due to *Uncinaria americana*.

Charleston, Charleston County.—Through the kindness of Drs. John Dawson and Robert Wilson, jr., I was able to meet the students of the Charleston Medical College. Explaining the object of my trip, I asked for volunteers to submit to microscopic examination. Sixteen of the students and 1 member of the faculty immediately volunteered. Of these 17 men (all white, of course), 4 were found infected with *Uncinaria americana* and 1 showed a heavy infection with

Hymenolepis nana. The cases of uncinariasis came from the sand districts—namely, Barnwell County; Florence, Saint John County; a sea-coast island near Charleston, and Edisto Island, Charleston County.

Through the courtesy of Dr. Huger and the ladies in charge of the Charleston Orphan Asylum, I was able to examine 230 white children, both boys and girls. I picked out 20 for closer examination, because of the anemic condition, or stunted growth, etc. Of the fecal specimens obtained, 15 showed infection with *Uncinaria americana*. All of the children came from sandy districts of the State—namely, Summerville, Dorchester County; Berkeley County; Adams Run, Colleton County; Plum Island, Charleston County, and McClellanville, Charleston County. One additional case failed to show eggs in the feces, but the clinical history during early childhood seemed typical for uncinariasis (see p. 58, case of L. B.). Of the 4 remaining cases (2 from Charleston (city) and 2 from Edgeville), 1 Charleston boy 11½ years old and 1 Edgeville boy 11 years old showed infection with whip worms (*Trichuris trichiura*).

Besides the Charleston physicians mentioned above, I am indebted to Dr. Grange Simons, president of the State board of health; Dr. J. Mercier Green, city health officer, and several other gentlemen, for their kind cooperation in my work. Dr. De Saussure stated to me that he had found the eggs of *Uncinaria* in the stools of several patients.

GEORGIA.

Atlanta, Fulton County.—Learning incidentally that Drs. Claude A. Smith and H. F. Harris were continuing their studies on uncinariasis in Atlanta, I left the city without seeing any cases personally. No reason was apparent which made it necessary to confirm their work for this locality, and, furthermore, the territory belonged to them as local men who were carrying on their investigations at their own expense.

In connection with northern Georgia it may, however, be stated that according to Dr. Lamartine G. Hardman, a member of the Georgia legislature, a condition exists in Jackson County, Ga., which corresponds to what I found in Kershaw and Lancaster counties, S. C.

Macon, Bibb County.—Through the kindness of the local physicians I was able to examine two white orphan asylums in Macon.

In one of these, Dr. Clark (consulting physician) and I selected from among the 85 children 17 boys and girls for closer examination. The selection was made in the same manner as at Charleston, namely, because of the pale, weak, or otherwise poor condition. Microscopic examination showed 12 cases of infection with *Uncinaria americana*, 2 cases of infection with *Hymenolepis nana*, and 3 negative cases. The uncinariasis cases came from sandy districts, namely: Americus, Sumter County, 1; Buena Vista, Marion County, 2; Thomasville, Thomas

County, 1; Savannah, Chatham County, 1; and Waycross, Ware County, Ga., 1; and Wacissa, Jefferson County, 1; De Land, Volusia County, 4; and Liveoak, Suwanee County, Fla., 1. Both *Hymenolepis* cases came from Georgia.

At another orphan asylum, through the kindness of Dr. Little, the consulting physician, I examined 112 white children, all from Georgia. I selected 21 for closer study, and the microscopic examination of the feces revealed 17 cases of infection with *Uncinaria americana*, 1 light infection with *Hymenolepis nana*, and 2 negative cases, 1 of which was doubtless malarial. The 17 cases came from the following places: Baxley, Appling County, 1; Cordele, Dooly County, 1; Darien, McIntosh County, 1; Effingham County, 1; Johnson County, 1; Jones County, 4; Kinderlou Station, Lowndes County, 1; Lyon, Tattnall County, 1; Monroe County, 1; Richwood, Dooly County, 2; Sandersville, Washington County, 1; Waycross, Ware County, 2.

Four cotton mills were next inspected, some of the houses of the factory hands were visited, and about 25 or 30 cases of uncinariasis were found. Inquiry developed the important facts that the infected persons had come to the mills from the rural sand districts, and that in general their condition improved with their residence in the city. An examination of the premises, both of the mills and of the houses, did not indicate that the disease would spread.

At a negro school in Macon I failed to find a single case of uncinariasis which could be diagnosed symptomatically with even a semblance of confidence. In one case only did I even suspect the disease.

"Circus day" brought thousands of people into view on the streets, many coming in from the surrounding country. Several cases of probable uncinariasis were observed among the whites, but none among the negroes.

Besides the Macon physicians mentioned above in connection with the orphan asylums, I am under obligations to Dr. A. M. Burt, and especially to Dr. H. McHatton for their cooperation in my work.

Milledgeville, Baldwin County.—Through the kindness of Dr. T. O. Powell, superintendent, and Dr. M. L. Perry, pathologist, of the State sanitarium, I was able to see a large number of the patients. Extreme cases of anemia were conspicuous by their absence. Two patients were selected as possible cases of uncinariasis, the microscopic examination being left in the hands of Dr. Perry, who has kindly written me that it was negative.

Fort Valley, Houston County.—With the cooperation of Dr. M. S. Brown, a local physician, I found about 10 cases of uncinariasis near town within an hour's time. The clinical histories were so typical that it was considered scarcely necessary to make a microscopic examination; nevertheless this was done in one case with the result of finding a severe infection with *Uncinaria americana*. After seeing the class of cases I desired to find, Dr. Brown assured me that they

were common in that region, and that he could easily find 50 or more cases within a day's time.

Albany, Dougherty County.—Leaving the sand district I next visited Albany, which is surrounded by clay. Corresponding to the change in the soil there was a change in the medical facies. Uncinariasis disappeared, except for cases which came in from the neighboring sand districts, while malaria increased. Dr. Hilsman, one of the local physicians, kindly drove around with me to find cases, but we were obliged to go about 15 kilometers (9 miles), namely, into Lee County, before we located a family with uncinariasis. During this drive we left the clay soil and passed into a sand district, with pine woods. The family in question presented four typical extreme cases; although microscopic examination for sake of diagnosis seemed unnecessary it was nevertheless made and showed heavy infection with *Uncinaria americana*.

While with Dr. Hilsman in Albany I made a most fortunate mistake in diagnosis. The patient was a boy about 13 or 14 years old. He had a clear case of malaria (typical history, enlarged spleen, etc.), but in addition to that I was confident that he had a medium or light infection with uncinariasis. Microscopic examination proved me in error. This boy lived in a clay district and had never lived in sand; furthermore, he did not show the eye symptom, which I shall discuss later (see p. 65). This is the last time I attempted a definite diagnosis upon symptoms of any medium or light case unless severe cases occurred in the same family.

As soon as Dr. Hilsman understood the kind of cases I desired to see he stated, with the utmost positiveness, that they did not arise in and around Albany until the sandy soil was reached. Occasionally cases came to Albany from the neighboring sand counties, but the local anemia Dr. Hilsman considered to be almost entirely of malarial origin—an opinion in which I can only concur after what I saw in that city.

I happened to be in Albany on Saturday, when the country folks for miles around come to town. Standing on the street corner for several hours, I must have seen about 200 whites and fully 3,000 negroes who drove or walked into town. Of the whites, I noticed about 5 cases of probable uncinariasis. Two of the cases, whom I was able to interrogate, gave a typical history of uncinariasis extending back for several years, but no history of malaria. They came from neighboring sand counties. Among the 3,000 negro men, women, and children, whom I saw, there was only one person in whom I even suspected from his general appearance that uncinariasis might be present.

Willacoochee, Coffee County.—In southern Georgia, Coffee County bears the reputation of being more or less a center for dirt-eaters. It is a sandy, pine-wood district, with numerous swamps, which indicate a more impervious subsoil. As a result, both malaria and uncinariasis

were found, and it was exceedingly interesting to note the ease with which an error in diagnosis in medium and light cases could be made if the microscope were not used. Extreme cases, however, could be easily distinguished without the microscope. Several cases of typical extreme uncinariasis were seen on the street, but not examined carefully. Then, through the kindness of Dr. Wilcox, I was able to make a careful examination, both physical and microscopic, of a group of 8 cases at the sawmills a short distance from town. After Dr. Wilcox examined these cases with me, he declared that he knew of at least 200 similar patients within the territory of his practice. He considered this condition one of the most common diseases of that region, but thought malaria was fully as common if not more so.

Waycross, Ware County.—Passing now to Waycross, I entered a district where uncinariasis is exceedingly common. Drs. R. P. Izlar and J. L. Walker assured me that the cases I was tracing, two of which I saw with Dr. Walker, were much more common than was malaria, the proportion being about 20 to 1. In this district these patients are called "Branch-water people." Both Dr. Izlar and Dr. Walker stated that they could easily show me scores of cases within a radius of a few miles.

FLORIDA.

Jacksonville, Duval County.—In Jacksonville two cases of typical uncinariasis were seen on the street. One of these patients was traced to the county in which the boy had formerly lived.

Both the State and the city health offices assured me that the condition I was hunting was perfectly familiar to them—that it was prevalent throughout the State, especially in the "flat-woods district," and that Florida physicians interpreted it as an anemia due to malaria and improper diet.

Waldo, Alachua County.—Through the kindness of the local physician, Dr. J. W. Boring, I was able to examine two groups of typical cases of uncinariasis within a short distance from town. Dr. Boring assured me that this condition was exceeding common in Florida and was generally interpreted as an anemia due to malaria and improper diet.

Ocala, Marion County.—In Ocala I saw several typical cases of uncinariasis on the street, and, through the kindness of Dr. A. L. Izlar, I was able to examine 5 positive cases and 1 probable case more closely. Dr. Izlar confirmed the statements of the health offices in Jacksonville, Fla., relative to the frequency and interpretation of the disease.

From Ocala I returned (November 16) directly to Washington, D. C., and noticed several cases which presented the appearance I had found typical of uncinariasis, as the train stopped at various stations in Florida.

SYMPTOMATOLOGY OF UNCINARIASIS.^a

In connection with the symptoms, let us recall that uncinariasis is caused by hookworms about half an inch long which live in the small intestine for several meters below the stomach.

These worms fasten to the mucosa and suck blood. They lay numerous eggs, which can be found by a microscopic examination of the stools. The number of eggs in the feces and, in a general way also, the severity of the symptoms will vary with the number of parasites present and with the duration of the infection.

The injury to the patients results from the following factors: (1) Sucking of blood by the parasites, which is a constant drain on the system; (2) loss of blood into the intestine through the minute wounds made by the parasite, a factor which also tends to deplete the system; (3) the wounds form points of attack for bacteria, hence increase the chances of bacterial infection as well as of toxic infection from partly digested and decomposed food; (4) the wall of the duodenum and jejunum becomes thickened and degenerated, and its function is thus decidedly interfered with; (5) the parasites in all probability produce a poisonous substance which acts upon the patient.

Theoretically there is only one sign which is present in every case, namely, the presence of one or more parasites in the intestine. If

^a This discussion of symptoms will be influenced to no slight degree by the fact that during my trip my associates have been almost entirely practicing physicians, particularly in rural districts, rather than laboratory specialists; and, since it is more particularly the country practitioner whom I desire to reach by this paper, I shall not hesitate to use vernacular names even if these do not invariably have a classical origin.

My trip was undertaken in order to prove the frequency and geographic distribution of the parasite, not to study the symptoms it causes. It was therefore a zoological, not a clinical, trip, and on this account a zoologist, not a clinician, undertook the investigation. Not posing in any sense of the term as a clinician, I feel that any observations which I have made upon symptoms, *sensu stricto*, should be looked upon as over and above the amount of work which should be justly expected of me. Certain symptoms I could not help noticing. The circumstances of my trip, the rapid travel, short stops, and the fact that the work was done among strangers, and usually in the field instead of in a hospital, absolutely excluded certain observations, even had I considered that I was the proper person to make them.

If, therefore, the reader misses in this discussion observations on any particular symptom in which he is especially interested, I beg that he will recall that it is self-understood that the finer points in symptomatology must be studied by expert clinicians.

I regret that it is not feasible for me at the present time to review the entire medical literature on uncinariasis. Such an undertaking would involve an unjustifiable delay in sending much-needed information to physicians in the infected district.

In connection with my own observations, however, I shall make frequent references to the noted paper entitled "Observations on 400 cases of anchylostomiasis," published in 1894 by F. M. Sandwith, M. D., physician to the Kasr-el-aini Hospital, Cairo, Egypt, thus supplementing my description with the views expressed by a trained clinician.

these worms are in an egg-laying stage ova will be found in the feces. But from a practical standpoint, severe cases present what seems to be a characteristic type, and even medium cases often present a more or less typical clinical history.

The statement is not infrequently made that there is one way and only one way to diagnose a case of uncinariasis, namely, by examination of the stools to find the parasites or their eggs.

Academically this statement is more or less correct, yet practically



FIG. 42.—A severe case of hookworm disease observed in Florida. Note the bloated face, the drooping shoulders, the prominent abdomen, and the thin arms and legs. This girl is about fifteen years old. Original, from a kodak photograph.

it should be somewhat modified. Sandwith (1894, p. 13), to quote from a clinician, states that “facies of the patient is characteristic, though it is difficult to describe his discontented, harassed expression, which sometimes changes to a ready smile after a month’s stay in a hospital.” The data at my disposal would lead me to divide the cases of hookworm disease I have observed into three general but not very sharply defined classes, namely:

(1) *Light cases*, including those in which practically no distinct symptoms of the disease are noticed, but in which a few hookworm

eggs are discovered in the stools. We may also place here a number of cases in which, in addition to the presence of eggs, there is a slight diarrhea or some other slight symptom, including more or less rapid exhaustion after physical exertion, hence an indisposition to work, which is usually interpreted as laziness. Cases of this class are found in the infected areas and elsewhere, since infected people may move away from the sand districts.

(2) *Medium cases*, including those in which the disease has progressed to such an extent that a more or less anemic condition is noticed, but other symptoms are not especially marked. If these patients were found outside the infected area, the diagnostician (especially if he is not familiar with the disease) would probably not see anything particularly characteristic in them; many of these cases, however, show a more or less typical history, and if a history of residence upon sandy soil in tropical or subtropical regions can be obtained, uncinariasis should certainly be strongly suspected. If these cases occur in a family which also presents severe cases of uncinariasis, the diagnosis of hookworm disease in the medium cases is usually quite safe, even if a microscopic examination is not undertaken.

(3) *Severe cases*, in which we find that striking set of symptoms which even the laity in our Southern States attributes to "dirt-eating." These patients present a facies which is well recognized by Southern physicians. If the patient is found in a Southern sand area, the diagnosis is practically certain. If found outside of the infected area, with a history of previous residence in a Southern sand district, its recognition symptomatically ought not to be attended with difficulty. In case of doubt, if a microscope is at hand, the test may be made in less than five minutes; if no microscope is at hand, the blotting paper test (see p. 81) will usually suffice.

Turning now to an analysis of symptoms, I will give my observations on the severe cases. It is needless to state that the symptoms discussed may vary in intensity, and that not every symptom mentioned is found in every severe case. We find on the contrary an imperceptible gradation between the severest and the lightest cases.

GENERAL PREDISPOSING FACTORS.

INFECTION OCCURS CHIEFLY ON SANDY SOIL.

In connection with the clinical history, the residence on sandy soil is undoubtedly one of the most important points to be obtained. If an anemic patient gives no history of temporary or permanent residence on a sandy soil, uncinariasis is not absolutely excluded, but according to my experience the chances are against it. If on the other hand a history of sojourn or residence in a sandy rural district is obtained, the probabilities of uncinariasis are decidedly increased.

Nearly every case of the disease found during the entire trip was either living at the time in a sandy district or had lived in such a place a few years previously. As soon as I entered the sandy areas, uncinariasis was found. As soon as I left the sand, as at Albany, local foci of infection of uncinariasis disappeared.

Inquiry among physicians at first failed to elicit any definite statement regarding the soil on which anemia was most frequent, but upon going farther south several physicians were met whose experience fully confirmed my observations on this point. One physician in particular, Dr. A. M. Burt, of Macon, was of the emphatic opinion that in bringing the condition which I have interpreted as uncinariasis into connection with the sandy soil, I had found the keynote to the distribution of the entire disease. Dr. McHatton, of Macon, called my attention to the fact that in antebellum days the slave owners in the Lower Mississippi Valley frequently provided special quarters, which were removed from the sand districts, and to which they sent the dirt-eating and other sick negroes and also negresses about to be confined, the view being held that a clay soil was more salubrious than a sandy soil. This view, in fact, I found to be rather prevalent among the farm hands. Time after time they remarked, "We were never sick so long as we lived in a clay district," "This disease developed after we moved upon sand," etc.

This view that uncinariasis follows the sand is supported by evidence obtained in Alaska by Mr. F. A. Lucas. After the discovery was made that uncinariasis, caused by *Uncinaria Lucasi*, was prevalent among the seal pups, Lucas (see above, p. 39) showed that it was practically only the seals on the sandy rookeries which were infested with the parasites.

As stated above (p. 39), I have also observed two outbreaks of uncinariasis among sheep and goats, caused by *Uncinaria trigonocephala* (Rudolphi, 1809) Ralliet, 1900, on more or less sandy soil, and have further confirmatory facts in connection with one outbreak of the disease among dogs. Since returning from my trip I have found at least one reference in literature on uncinariasis and ground itch to the effect that the soil in districts where certain cases have occurred was more or less sandy (see p. 62), but I have not yet found that any author lays stress upon this point.

In view of all the data at hand, I have no hesitation in expressing the opinion that uncinariasis, caused by *Uncinaria americana*, is pre-eminently a disease of sandy localities and that cases found in clay or rocky areas can usually be traced to a former visit or residence in a sandy place.

Just why this disease should follow the sand rather than the clay is not absolutely clear. Three explanations have occurred to me as working hypotheses which, though not absolutely satisfactory as final, will, I believe, explain part of the mystery.

(1) We know that uncinariasis is spread through the feces; we know further that when the embryos hatch from the eggs they leave the feces and enter the surrounding water or moist earth, while there is no satisfactory evidence to show that they are blown around in the air in a dry state. (See Stiles, 1902b, p. 199.) Now, assume that a person walks over infected ground; if that ground is clay, he does not disturb the embryos which have crawled^a beneath the surface, except in wet places; if, on the other hand, the ground is sand, he not only stirs it up while walking, thus bringing the young worms nearer the surface again and thereby increasing their chances of producing an infection, but he is also likely to carry away particles of sand, together with embryos, with him on his shoes or feet, thus increasing his chances of becoming infected. It is further clear that children playing in sand will stir up more embryos than when playing on a clay soil, and will thus increase their chances of infection.

(2) An additional explanation is that water will not pass through clay as it will through sand; hence on clay soil the embryos stand a greater chance of perishing or of being washed by rain into the streams. On sand, however, the embryos might perhaps work their way through the soil^a with the water, and thus infect surface wells. In advancing this hypothesis, I am not unmindful of the view, supported by excellent observers, that, since the embryos sink in water, drinking water is not necessarily a common source of infection. Granted that they do sink in water, a water bucket in a well also sinks, and the water from surface wells frequently contains sand particles that are heavier and larger than *Uncinaria* larvæ; hence we can not altogether ignore the drinking water as a possible source of infection. If, on the other hand, drinking water were the only source of infection, it is probable that in families where uncinariasis exists the intensity of the disease would show a greater tendency to uniformity.

Giles has examined 56 specimens of water from wells and ponds of villages affected with hookworm disease and 16 of these he found by chemical and microscopical examination to be "bad" or "very bad." Yet on only one occasion did he find a rhabdite of doubtful origin in water. (Sandwith, 1894, p. 9.)

(3) Oxygen is necessary to the development of the embryos and larvæ, and it does not seem unreasonable to assume that sand would

^a In an article which has just appeared, Looss (1903, p. 331) says:

"Further, during the six years of my residence in Cairo [Egypt], I have not heard, up to the present time, of a single case [of hookworm disease] in an European (my own case, of course, excepted). This fact speaks all the less [so much the less] in favor of the assumption of a more common dissemination [i. e., infection] of the disease through the drinking water, vegetables, etc., since [as] *the mature hookworm larvæ, as experiments have shown, pass through the ordinary sand filter with surprising rapidity, and this even when the water is allowed, not to run off, but to stand.*" [Italics not in the original German.]

on that account present more favorable conditions for the growth to the "encysted" stage, and probably also a longer preservation of that stage.

In this connection it may be noted that Looss has used charcoal as a medium in which to cultivate the larvæ of *Agchylostoma duodenale*.

INFECTION OCCURS CHIEFLY IN THE RURAL DISTRICTS.

Since the infecting agent of uncinariasis is spread through the feces, we may expect to find infection taking place (other things being equal) in localities where the fecal matter is not properly disposed of. Furthermore, we would not expect that paved streets or grass lawns would favor the development of the disease. We need not therefore expect local foci of infection to occur in cities and towns which have proper sewerage systems and in which the streets and walks are paved and the yards sodded; but we may expect to find local foci of infection in localities where box privies are used but not properly cared for, or where promiscuous defecation occurs in the woods, fields, mines, etc. This condition is in fact exactly what is found. While uncinariasis is not entirely absent from those premises on which the feces are properly disposed of, in cities like Washington, Richmond, Charleston, Macon, Jacksonville, and Ocala, such cases as are found can not be shown to have developed there; but probably in most every case they can be either probably or positively shown to have been contracted elsewhere. In a number of cases I have established this point with a probability, which for all practical purposes may be looked upon as a certainty. We may therefore exclude the greater portion of the inhabitants of sanitary districts of cities from consideration in connection with uncinariasis and may with confidence lay down the general rule that any anemia developing in them as result of local infection (namely infection at home) is much more likely to be due to malaria or other causes than to uncinariasis.

As we approach the outskirts of cities and towns and enter the rural districts, localities in which box privies are used but not always properly cared for, we meet with conditions which are more favorable to infection with the hookworm disease. Accordingly, in cases of anemia, especially in women and children, developing in such localities, uncinariasis must be taken into consideration as one of the possible causes.

Sandwith (1894) states that his patients came from all parts of Egypt except some of the seaport towns.

As we go into the country and visit the farms we not infrequently find a condition, in respect to the disposal of fecal discharge, which almost beggars description. Taking the rural districts visited during my recent trip as example, it is not an exaggeration to say that with the exception of the planters' premises, not over half of the country houses or huts of the sand regions have any privy at all; if there is

one present, it is rarely properly cared for; furthermore it is the exception rather than the rule that it is used. As a result uncinariasis is widespread, not because the country air is particularly favorable to its development, but simply because so little attention is paid to the proper disposal of the fecal discharges.

SYMPTOMS ARE MORE SEVERE IN SUMMER THAN IN WINTER.

It is almost universally conceded that the patients are in better condition in winter than in summer. According to testimony, the symptoms begin to increase in the spring and to decrease in the early winter. This periodicity will be noticed, of course, only in localities which are above the frost line, and it is easily explained when we take into consideration the biology of the parasites. Cold retards and heat hastens the development of the eggs and the embryos; a freezing temperature of 24 to 48 hours' duration, it is said, kills both eggs and embryos. Accordingly, after frost sets in in the fall, the patients will add less to the infection which is present in their bowels than they will during the summer. Some of the worms already in the intestine will be passed, thus decreasing the number of parasites present; the patient will accordingly lose less blood and will on this account feel somewhat better. As warm weather begins in the spring the free eggs and embryos will develop more rapidly and the infection will be increased. There will be more parasites in the intestine, hence symptoms will be augmented.

Some few patients, however, insist that they are better in summer than in winter.

It is probable that the seasonal periodicity of the symptoms noticed in our Southern States will be modified in the Tropics, so that the symptoms will increase in severity in the rainy season and decrease in the dry period of the year. Such a periodicity would correspond to the biologic fact that the eggs and embryos perish very quickly upon becoming dry.

In patients who are not subject to continued infection, as for instance those who have left the area of infection, the seasonal periodicity may be expected to disappear.

WHITES APPEAR TO BE MORE SEVERELY AFFECTED THAN NEGROES.

Osler and other observers have already noticed that chlorosis is more frequent in blondes than in brunettes.

Uncinariasis occurs in both blondes and brunettes, and in both the white and the negro, but so far as my observations go the disease is more severe, *or at least more noticeable*, in blondes than in brunettes, and much more severe, as a rule, in the white than in the negro. This observation was supported by all the evidence I could gather from local physicians. In fact, several practitioners declared that they had never seen a case in the negro to recognize it. There is, however, abundant evidence that such cases do occur.

I am at a loss for a satisfactory explanation of the comparative freedom from uncinariasis noticed in the negro. They live under the same conditions as the poorer classes of the white population, except that, as a rule, the negro farm labor is more common in the rich than in the sand districts; however, negroes also live in infected districts. It is true, as frequently claimed, that some of the negro habitations are more clean than some of the homes of the poorer whites. Still, not all negro huts are cleaner than all white huts. The personal habits of the negro children are certainly no more hygienic than those of the white boys and girls.

One factor which may possibly play a rôle in this comparative freedom from the disease on the part of the negro is the fact that negro women very frequently give their children "worm tea," made from certain plants, in order to expel the "eelworm" (*Ascaris lumbricoides*), or they give calomel "to regulate the liver," and this may perhaps also result in expelling the hookworms before the latter have had an opportunity to do much harm. Still this explanation is not altogether satisfactory, especially in view of the testimony of both the local physicians and the negroes themselves to the effect that eelworms are much less common now than formerly, hence "worm tea" is not taken so frequently as in former years.

Chabert (1821a), Jordan (1832), Imray (1843), Le Conte (1845), Duncan (1850), and other authors report "dirt-eating" among the negroes. In fact, nearly all early authors who describe "dirt-eating" lay special stress on the frequency of the habit among the slaves.

In conversation with a negro druggist, I was informed that while dirt-eating was formerly said to be more or less prevalent among the negroes, it was an acknowledged fact among them that the custom had greatly decreased in recent years. In connection with this statement we may note the interesting claim by Cotting (1836a) that there was a reduction in dropsy and dirt-eating corresponding to the more general use of calomel.

Sandwith remarks upon the apparent comparative freedom of the negro from uncinariasis and anemia. Zinn and Jacoby (1896) also refer to the frequent presence of *Agchylostoma duodenale* in negroes of Africa, in whom the anemia was not prominent.

Can it be that the poison produced by the hookworms has less effect upon negroes than on whites and that on this account the disease is less severe in the dark races?

OCCUPATION OF PATIENT.

It is generally acknowledged by writers on uncinariasis that the disease is especially prevalent among people who in their daily work come in contact with earth. In fact, the malady is sometimes called "brickmakers' anemia" or "miners' anemia." Sandwith mentions "peasants" as apparently forming the majority of his patients.

In my own work I was surprised to find that hookworm disease was comparatively light and comparatively rare among the miners I examined. Of brickmakers my statistics are too small to permit any generalization. Two examinations out of eight showed light infections. Most of my cases were from the farming classes.

Sandwith emphasizes the fact that his patients came from the poorest class of the community, and that of 200 men 190 were accustomed to work with their hands in more or less damp earth. Of these 190 men, 152 were agricultural laborers; 18 were masons or bricklayers' laborers; 7 were "scavengers of street refuse and of cesspools, accustomed to emptying with their hands the dry contents of the latter;" 7 were peddlers of unwashed vegetables; 3 limestone carters and scavengers; 1 gardener; 1 fisherman in the mud; 1 "shadouf" worker at the Nile bank; 1 coffee-stall keeper; 3 readers of the Koran; 1 blacksmith; 1 shoeblack; 4 beggars. Sandwith also mentions 20 cases among policemen, who dated their illness from periods of life when working as agricultural laborers.

SEVERE CASES ARE MORE COMMON IN WOMEN AND CHILDREN THAN IN MEN OVER TWENTY-FIVE YEARS OF AGE.

The assertion is frequently made that uncinariasis is more common in men than in women and children. This statement may be perfectly correct in mines and brickyards, but it does not hold good for the farming localities I visited, where the greater prevalence of severe cases in children than in adults, and in women and children than in men over 25 years of age, is very striking. The conditions found, in respect to this point, seem to be due to four factors in particular, namely: (1) The average family in the country districts numbers from, say, 6 to 12. As a rule, 2 or 3 of these can be called adults, and 3 to 9 can usually be classed as children (including minor boys and girls). Since there is a greater number of children than adults subject to infection, we should naturally expect to find a greater number of cases among the children, and our expectations are fully realized. (2) The fact that children and women present a greater number of cases than do the adult males over 25 years of age I am inclined also to explain on the ground that the former are at home more than are the men; the area immediately surrounding the house, for, say, a radius of 50 meters, is a more common place for defecation than are the more distant fields, hence it is more severely infested with the infecting agent. Now, while it is true that, among certain classes, both women and children work in the fields, it is also true that they do this much less than the men. They are at home more, therefore they are on the more intensely infected area for a greater length of time; hence, in respect to actual time they are more subject to infection than are the men, and, other things being equal, they will present a greater num-

ber and a greater proportion of extreme cases. (3) The children in playing in the sand around the house, and owing to their more careless personal habits, are of course especially liable to infection. (4) The men being stronger, are, as a rule, better able to withstand the effects of infection. An interesting and important fact is that men of about 20 to 24 seem in many cases to more or less outgrow the effects of the malady. They give a history of medium or severe infection from, say, 10 to 18 years of age, then at 18 or 20 they begin to improve, and finally appear much better, although their features and physique still show the effects of former disease. This time of improvement corresponds to the years following their first more active participation in work which takes them more away from the house, hence to years when they are subjected less constantly to infection.

Sandwith states that nearly all of his 402 patients were in the prime of life, between 20 and 40 years of age. His youngest case was a boy of 6 years; 48 patients were between 10 and 20 years of age; 170 patients were between 20 and 30 years of age; 140 were between 30 and 40 years of age; 21 were between 40 and 50; 15 were between 50 and 60; and 7 were above 60.

Giles reports a case of a girl 4 years of age. My youngest patient was 3 years old.

Of Sandwith's 402 cases only 3 were females. This statement is in striking contrast to my observations. Sandwith and I made our studies, however, under totally different conditions, for his patients came to his hospital, while I went to the homes of the infected persons. Possibly this will explain, in part, our different results, for he states that "women have not yet learned to apply for hospital relief in the same proportion as the men."

SEVERAL CASES ARE LIKELY TO OCCUR IN THE SAME FAMILY.

Uncinariasis commonly occurs in groups of cases. If one child in a family is accused of being a "dirt-eater," and is shown by physical and microscopic examination to represent an extreme case of uncinariasis, an examination of the remaining members of the family will usually show that most or all of them are suffering from anemia in different degrees, and the microscope will disclose infections with *Uncinaria*. This condition of affairs is so general that it may be looked upon as the rule among farming classes, while the occurrence of isolated cases in a family, except possibly among miners, may be looked upon as the exception. Numerous observations could be cited in support of this view; for instance, in the first family examined in the sand district near Haile Goldmine only one member had the reputation of being a "dirt-eater," but all 11 were in different stages of anemia. In a family of 10 members on a neighboring plantation only 1 was noted as a resin-chewer, but all 10 were anemic. In orphan

asylums it was noticed that if two or more children of one family were present and one child was affected, the other children were also affected. In fact, in every instance where I was able to examine the family to which an alleged "dirt-eater" or "resin chewer" belonged, all or nearly all the members of the family showed an anemia with the general history of uncinariasis, and in every case examined microscopically the eggs were found.

Two families may, however, live very close together, and one family may show several severe cases while the other may not show a single case severe enough to be suspected symptomatically. Thus, on one plantation all the hands examined showed uncinariasis, while the planter's family, of much more cleanly personal habits, did not exhibit any signs of infection. It is, however, more common to find that where one family is affected other families of the same neighborhood will show infection, provided of course that the infected family in question has not moved into a city or a clay district.

The occurrence of cases of uncinariasis of different degrees of intensity in family or neighborhood groups is easily explained by the fact that in a given family or neighborhood all persons are subject to the same general conditions of infection, but owing to differences in age, daily occupation, personal habits, etc., some will be more subject to infection than others.

OBJECTIVE AND SUBJECTIVE SYMPTOMS; ANALYSIS OF SYMPTOMS.

It is difficult to draw a distinct line between the subjective and the objective symptoms. If a patient is left to tell his own story, practically all that the observer will learn is that the patient "feels weak, has a headache, gets dizzy, has fluttering of the heart, finds it hard to breathe, feels worse in summer than in winter, and has 'misery' in the 'stomach.'" In medium cases a few judicious questions, directed more to the parents than to the patient, will as a rule bring to light a history upon which, taken in connection with what one can himself observe, a probable diagnosis may be made. As a rule, little weight can be placed upon the statements made by a patient suffering from an severe infection of uncinariasis. He will answer "yes" or "no?" in a most contradictory manner, so that by putting questions in different ways it is practically possible to make him admit or deny, as desired, any particular symptom. It is chiefly from the patient's family that one must judge of what the person has complained.

ONSET AND INCUBATION.

As it takes the parasites four to six weeks to reach maturity after entering the system, the earlier symptoms will be more particularly gastro-intestinal (see, however, p. 60), and even if these are present in a marked degree we can not look for a diagnosis by microscopic

examination of the feces until the worms begin to lay eggs. It is, however, not excluded that some of the young worms might be passed in the stools and be identified, though such a chance is probably more theoretical than practical. In an experimental case of infection through the skin, Looss showed that eggs did not appear in the feces until 71 days after infection. According to Sandwith (1894, p. 12), Surgeon-Major Giles suspected that many of his patients in Assam had suffered from fever at the onset of their malady, and he was confirmed in this impression by observing pyrexia in the monkeys fed on hook-worm embryos.

Looss (1897, pp. 914-915) noticed nausea as the first symptom in dogs to which he fed the larvæ of *Agchylostoma duodenale*.

Bentley (1902 a) practically advances the view that "ground itch" is the primary symptom, and since Looss's view of infection through the skin is correct, at least for some cases, some cutaneous symptom must in such instances be the first symptom of uncinariasis. (See p. 60.)

STAGES OF UNCINARIASIS.

Since my observations of each patient were confined to one or two days, they would not justify me in dividing the disease into stages. Lutz (translated by Macdonald) recognized the following stages:

I. STAGE OF PURELY LOCAL SYMPTOMS.

(a) *Acute form*; (b) *chronic form*.—The symptoms are similar in both forms. The disease is yet limited to pains and disordered digestion; no pallor, no rise of pulse.

II. STAGE OF SIMPLE ANEMIA OR OLIGOCYTHEMIA (CHLOROTIC STAGE).

(a) *Acute form*.—1. Slight degree: Conjunctival vessels still visible; nails and lips pale red; pulse increased in frequency; no blowing murmurs over cardiac area.

2. Higher degree: Conjunctivæ devoid of vessels; nails whitish; lips pale; pulse frequently very much increased; no blowing murmurs.

(b) *Chronic form*.—Anemia has not reached the highest degree. In many cases distinct cardiac hypertrophy and dilatation; in other cases disordered valve closure; seldom both combined. Moderate increase of frequency of pulse.

III. DROPSICAL STAGE.

(a) *Acute form*.—A high degree of anemia; pulse small, much increased in frequency; no blowing murmurs; edema of a hydremic character.

(b) *Chronic form*.—Symptoms of cardiac defects, with disturbed compensation, or of fatty degeneration; distinct symptoms of cyanosis; droopy of engorgement; anemia of varying intensity; disordered nutrition.

DURATION OF UNCINARIASIS.

In speaking of the duration of uncinariasis, we should clearly distinguish between the duration of cases which remain in the infected areas, and the duration of cases which after once becoming infected move to uninfested districts where conditions are such that reinfection is excluded.

In the former instance we have to deal with cumulative infection, taking place week after week and year after year, hence successive generations of parasites come into consideration.

In the latter instance we have to deal with the individual life of the parasites which are present in the patient at the time he leaves the infested area.

A failure on the part of most observers to distinguish between these two classes of cases renders the published data less valuable than they otherwise would be in determining the duration of uncinariasis.

In my own observations, I have found people remaining in the infested areas who gave a history of the disease extending back for ten, twelve, and even fifteen years. I have further three observations which I consider free from criticism in connection with patients who presented the disease six years (2 cases) and even six years and seven months (1 case) after being removed from the source of infection. I also have one observation, which is not free from criticism, upon a patient who showed the disease ten years after entering a hospital.

Sandwith (1894, p. 15) in discussing the duration of illness says—

“Nearly every patient said he had been ill two or three years before admission. I find among my notes 29 cases, uncomplicated by other diseases, where the patients said they had suffered more than three years. Of these, 13 had been ill for four years, 6 for five years, 3 for six years, 2 for eight years, 2 for nine years, 2 for eleven years, and one vowed it was fifteen years since the commencement of his symptoms.

“It may be safely said that during these long intervals of time very few patients had any rational treatment.”

It is probable that Sandwith's cases were subject to cumulative infections.

LENGTH OF LIFE OF THE INDIVIDUAL PARASITES.

It is already established that for every hookworm which is present in the intestine a separate embryo must enter the body. In other words, the eggs which the female worm deposits in the intestine will not develop there to mature parasites, but must first be discharged in the feces and undergo certain changes.

It is further clear that direct autoinfection, such as takes place in the case of pinworms (*Oxyuris vermicularis*), is excluded. For instance, suppose a child is at stool and soils his fingers with the feces, which contain hookworm eggs, then puts his fingers into his mouth and swallows the eggs; these ova will not develop in the bowels into adult worms.

Whether an *Uncinaria* egg, which happened to get under the finger nails, could reach the larval infecting stage in that place is perhaps an open question. So far as I am aware, the point has never been studied, but what is already known about this group of parasites does not lead me to believe that such a condition would be especially common, although it does not seem absolutely impossible.

With the foregoing premises in mind, it is important to determine how long the parasite in the intestine can live. Regarding the American hookworm, *Uncinaria americana*, I can present the following data: Of children at the Charleston Orphan Asylum in whom I demonstrated the presence of *Uncinaria americana* microscopically, it may be noted that 8 children had been in the orphan asylum two years or less; 4 children had been in the asylum between two and three years; 2 children had been in the asylum six years; 1 child had been in the asylum six years and seven months.

I have selected the Charleston Orphan Asylum as best fulfilling the conditions desired to illustrate the point at issue. The refined discipline, the scrupulous cleanliness, and the general hygienic conditions noticed are such that local infection is practically excluded. It must be admitted that some persons coming in from the country might possibly bring on their shoes a few embryos, but such a theoretical possibility is altogether too remote to explain the 15 cases found.

For all practical purposes we are justified in assuming that the hookworms which these 15 children had in them when I saw them were the same individual worms which were in the children when the latter entered the asylum, and from the data obtained it is clear that hookworms of the species *Uncinaria americana* are capable of living six years and even six years and seven months.

A sixteenth child (case of L. B.) examined showed a clear clinical history of uncinariasis of long standing. Her condition at the time of entering the institution, as described to me by Dr. Huger, and her present complexion, eyes, stunted growth, and inferior mental development leave no practical doubt in my mind regarding the diagnosis. No normal eggs were found in her stools, despite the fact that I made 25 slides. One slide showed a single abnormal egg which had evidently been dried and had clung to the slide after an examination in some other town. I do not know this girl's complete therapeutic history during the past twelve years, but from the absence of eggs in her stools it is necessary to conclude either that the worms had been expelled by the drugs taken or else *Uncinaria americana* is not able to live twelve years.

Ashford (see Stiles, 1902b, p. 210) mentions a case where a boy "had been the host of the worm for probably ten or fifteen years," but he does not state that during this time the patient was not exposed to further infection.

The clinical importance of the length of the life of the parasite is self-evident. Suppose a physician in the North has an anemic patient, or a physician in the South has an anemic patient who lives in the city or in a clay district; it is not sufficient to inquire whether he or she has recently been exposed to malaria, but inquiry should extend for eight or more years back in order to develop the fact whether she has

during this time visited any tropical or subtropical sand area. If such a fact does develop, uncinariasis is among the probabilities and a microscopic examination of the feces should be made.

GENERAL EXTERNAL APPEARANCE.

GENERAL LACK OF DEVELOPMENT—STUNTED GROWTH.

In severe cases of long standing the patient is undeveloped both physically and mentally. A boy or girl 12 to 14 years old may be as small as the average child of 6 or 8; a young man or woman of 18 to 22 years old may present the general development of a child 12 to 16 years of age, but the face may appear either like that of a child or like that of a very old person, especially like that of an elderly dwarf. Similar conditions have been described as a symptom of dirt-eating.

SKIN.

The skin has an anemic, waxy white to a yellow or tan, shriveled, parchment-like or tallow appearance. In general it is that color known in the South as a "Florida complexion." In some cases of malaria, if one trusts too much to the appearance of the skin, he is likely to be misled into an erroneous diagnosis of uncinariasis, but the general clinical history is usually sufficient to distinguish between the two diseases, while the microscopic examination gives a positive diagnosis. (See also, Temperature, p. 72.)

Wounds heal slowly.—Among the first severe cases of uncinariasis found, I noticed that several of the patients wore bandages. Suspecting the possibility of ground itch and recalling Looss's and Bentley's theories (see below), I immediately inquired into the history of the sores. According to the testimony of the patients the ulcers present had started as small wounds produced mechanically; the wounds had failed to heal promptly, had grown worse, and were now about a year old. This same story was related to me by quite a number of patients, and many other persons suffering from uncinariasis assured me that in their cases cuts and bruises healed very slowly, testimony which was repeatedly corroborated by local physicians.

Cutaneous lesions caused by uncinariasis.—The statements just made lead to a consideration of certain views recently advanced by Looss and Bentley.

Looss's theory of cutaneous infection.—Looss (1901) has shown that if a drop of water containing embryos of *Agchylostoma duodenale* is placed upon the skin an itching sensation is produced; the worms enter the hair follicles, and from there they seem to bore into the surrounding tissues. Looss further advanced the rather startling opinion that the larvæ then reached the intestine, and he recounted observations which gave a certain amount of plausibility to this view.

More recently (see Sandwith, 1902), Looss has performed experiments which, so far as can be judged at present, demonstrate the correctness of his theory. According to Sandwith (1902), Looss^a smeared on the back of a puppy a mixture of charcoal and feces in which hookworm larvæ had been bred. Between nine and ten days afterward the puppy died and was found to have anemia of most of his organs, and a plentiful supply of young hookworms was found in his jejunum. A second puppy was treated in a similar way and also died on the night between the ninth and tenth days. Upon post-mortem he also showed exactly the same results. A man who offered himself for experiment was also similarly treated on his forearm, and in his case the first hookworm eggs were discovered in his feces on the seventy-first day. In all three experiments the feces were regularly examined for some weeks prior to the experiments, so that, so far as we can now judge, the results must be accepted, despite their very startling nature. Furthermore, Looss is known as too careful an investigator to permit any foreseen error to creep into his conclusion.

Somewhat similar results, namely the entrance of embryos into the skin, have been obtained by van Durme (1902, pp. 471-474) in experiments with *Strongyloides stercoralis* on guinea pigs, and it is needless to insist on the great importance of Looss's demonstration.

Bentley's theory of ground itch.—In line with Looss's views, Bentley (1902a) has made certain exceedingly important observations and experiments, the logical conclusion of which, taken in connection with Looss's work, seems to be that at least certain forms of ground itch constitute the initial symptom of uncinariasis. Bentley defines ground itch as follows:

Ground itch—Synonyms: *Panighao*, *water itch*, *water pox*, *water sores*, *sore feet of coolies*—is an affection of the skin, confined entirely to the lower extremities, and probably always associated with the presence of the larvæ of *Ankylostoma duodenale* in the soil of the affected areas; endemic in Assam and the West Indies and possibly present in other parts of the Tropics; characterized by its periodical epidemic appearance in the infected areas, coincident with the onset of the rainy season; with typical lesions consisting in a primary erythema, followed by vesicular eruption, which frequently becomes pustular and in severe cases may result in obstinate ulceration, or even gangrene.

Dr. Seheult (1900), of Trinidad, has suggested that the disease is probably due to some chemical irritant present in the soil, either natural or due to manure used in cultivation. Dr. Dalgetty (1901), of South Sylhet, struck by the resemblance which the lesions bear to scabies, and finding a mite present which he named *Rhizoglyphus parasiticus*, assumed this acarine to be the cause. These authors and Bentley (1902a) seem to agree that ground itch is a filth disease which

^aDuring the proof reading of the present report, Looss's (1903) article, detailing his brilliant experiments, has appeared. He demonstrates, beyond any question of a doubt, the correctness of his contention that infection with hookworms may take place through the skin.

is increased by the lack of care given to the proper disposal of alvine discharges.

Bentley (1902a) found in a water sore a young worm which he considered to be identical with *Agchylostoma duodenale*. He then performed the following experiments—

a. Some ordinary soil was sterilized by heat, and after being moistened with sterilized water, was infected with a small quantity of fecal matter containing numerous ova of *Agchylostoma duodenale*.

b. A similar preparation of soil was infected with a small quantity of feces, which on examination was found to be free from hookworm infection.

These two preparations were incubated at the ordinary temperature of the air for about a week, when sample “*a*” was found to be swarming with larval hookworms and various forms of bacteria and fungi, and sample “*b*” was similar in appearance except that no hookworm larvæ were found.

Each sample was then divided into two parts, *a'* and *a''* and *b'* and *b''*. Samples *a'* and *b'* were kept moist, while *a''* and *b''* were gently dried by exposure to the air for eight hours. Previous experiments had shown that six hours gentle drying at ordinary temperatures was sufficient to kill the hookworm larvæ. After remoistening *a''* and *b''* with sterilized water, the four samples were applied to the wrists of the subjects of experiment for eight to nine hours, and then they were removed. Fifteen hours after the first application, considerable erythema with a minute papular eruption appeared over the spot to which *a'* had been applied; within twenty-four hours a distinctly vesicular eruption had developed, followed by pustules exactly resembling those found in the lesions of ground itch. In the other cases a faint reddening of the skin was produced, which shortly afterwards disappeared. A reexamination of sample *a'* now showed that no live larvæ were present, although one or two dead worms were found. Sample *a''* still contained the dead larvæ. Apparently, therefore, the live larvæ *a'* had entered the skin and their entry had been followed by lesions similar to those found in water sore.

According to Bentley, also, it is probable that the acuteness of the inflammation attending an attack of the ground itch is largely governed by the nature of the organisms which accompany or follow the larval hookworms in their passage through the skin.

Regarding the treatment of ground itch, Bentley says that in the papular and early vesicular stage of the disease the application of a strong solution of salicylic acid in collodion or methylated spirit will cause the eruption to dry up, and so cut short the attack of the disease to one or two days. If, however, pus has formed, the only treatment of any service is the opening up and disinfection of the pustules with pure carbolic acid, silver nitrate, or nitric acid, and the after treatment of the sore as an ordinary ulcer. In cases attended with great swell-

ing, inflammation, and tendency toward the formation of sloughs, free skin incisions and the use of hot antiseptic footbaths are indicated.

Dalgetty (1901, p. 77) advises the application of a strong solution of lime and sulphur; strong phenyl solution is also beneficial, and a coating of coal-tar acts for a time as a preventative against infection; but when once the vesicles have formed pustules are sure to follow, and then the only remedy is to open them, evacuate the pus, and thoroughly cleanse them.

An anonymous writer (? Dr. Elliot, of Assam) in the *Journal of Tropical Medicine* (1900), gives the following directions:

"The indications are to get the case as soon as possible; to carefully cleanse the foot by soaking it in warm antiseptic solution; then open the vesicles with sharp pointed scissors, snip the loose skin away, and finally wash the parts with carbolic acid solution (1 in 40), and treat the resulting ulcer with carbolic acid, phenyle oil, extract of paroli leaf, zinc ointment, etc., according to circumstances. The soaking, washing, and dressing operations are repeated once or twice daily, and healing takes place in eight or nine days in favorable cases."

It will be noticed that Bentley does not definitely state that he adopts Looss's view of intestinal infection through the skin; nor does he definitely state that the hookworm larvæ act as anything more than carriers of bacteria. The conclusion would therefore seem to be that the ground itch with which he was dealing is a bacterial infection due very probably to fecal bacteria.^a If this interpretation is correct, its *dependency* upon uncinariasis does not seem to be proved, although its occurrence with the disease would seem to be established.

Additional facts (besides Bentley's experiments) which support the view that the ground itch, with which he was dealing, is more or less connected with hookworm infection are the following:

Ground itch occurs in the warm rainy season, especially in June, July, August, and September, and does not occur in cold weather, even when it rains; thus the seasonal distribution in general agrees with the infection period of uncinariasis. Grass-covered soil and smooth beaten roads do not cause it, neither does working in loose dry soil; and these conditions are unfavorable to the development of uncinariasis. The number of cases increases after a heavy rain and rapidly decreases during a hot spell, a fact which agrees with the biology of hookworms. "The soil itself is sandy, with clay here and there; a belt of pure sand, 40 to 60 feet thick, lies at a depth of 6 to 18 feet from the surface;" and uncinariasis is preeminently a sand disease.

Still the question is not quite so simple as would at first appear, and in connection with the subject the following points come up for consideration:

^aLooss has, however, proved that certain cutaneous symptoms follow the entrance of hookworm larvæ into the skin.

1. According to Bentley, ground itch is confined entirely to the lower extremities, and other authors state that it rarely extends above the ankles. According to the physicians in Georgia and Florida, it is found on other parts of the body also. I saw one case, said to be typical, where the disease was confined to one arm. Under these conditions is the panighao, discussed by Bentley, identical with the so-called "ground itch" which is so common in the southern portion of the United States, or is only a part of the American "ground itch" produced by hookworm larvæ?

2. If ground itch is the initial stage of cutaneous infection with uncinariasis (as Looss's and Bentley's views would seem to indicate), why should it be confined entirely to the lower extremities? If infection by uncinariasis frequently takes place through the skin, would not the hands and arms also, especially of children, and more particularly the soles of the feet and the palms of the hands, the spaces between the toes and fingers, and under the toe nails and finger nails, be the most common initial points of ground itch in case this latter is an initial symptom of uncinariasis?

3. On the same premises would we not commonly find lesions corresponding to ground itch on the abdomen of cattle, sheep, goats, dogs, cats, foxes, seals, and other animals suffering from uncinariasis? I will not deny that such lesions occur, but I have seen many cases of hookworm disease in certain of these animals and I have no recollection of having observed anything which corresponded to ground itch. If it were as prominent, in the animals named, as the typical "ground itch" of man which I saw in Georgia, I doubt whether I should have overlooked it. Possibly I did not have recent infections before me.

4. According to the testimony of Georgia and Florida physicians "ground itch" is exceedingly common; it occurs at some period in the life of practically every person, unless he lives exclusively in the city; it occurs in the healthy as well as in the sickly, and in persons who neither at the time of infection nor later show the slightest evidence of anemia. These statements, which I have repeatedly heard from Southern physicians, can not be said to indicate that Bentley's views are applicable to all cases of "ground itch" as we find this disease in this country.

5. "Ground itch" is said to be common in clay districts as well as in sand districts. Under these circumstances, why is uncinariasis so pre-eminently a disease of the sand areas?

6. Bentley states that "ground itch" is probably always associated with the presence of the larvæ of *Agchylostoma duodenale* in the soil of the affected areas. Doubts may, however, arise as to whether a sufficiently wide geographic range has been examined in connection with this point.

In view of the above considerations, it will be well to remain open

to conviction awaiting a more thorough demonstration of the broad application of Bentley's interesting and valuable views. But until better proof is advanced than has thus far been brought to my attention, I find it impossible to unreservedly adopt the opinion that American ground itch is *necessarily* connected with uncinariasis.

While not opposing the theory of infection through the skin, but admitting, on the contrary, that Looss has proved his point, I may state that the conditions which I saw in the southern portions of the United States do not indicate that any indirect method of intestinal infection is necessary in order to explain the severe cases of uncinariasis observed. The average boy or girl suffering from this disease is not conspicuous because of personal cleanliness. Bath tubs are not found in their homes, and from physical examinations I made I can testify that not only their hands and finger nails, but their entire bodies also, are far from a condition unfavorable to parasitism. Sucking the fingers, picking the teeth, biting the finger nails, or even eating a piece of bread with soiled hands will usually suffice to convey some dirt between the lips. The sand on which the children play must be heavily infested with hookworm larvæ, and it certainly can not be an exceptional occurrence that the children unconsciously carry microscopic worms to their mouth. Further, the chances for infection of surface wells, from which the drinking water is taken, are very great in any sandy soil. If, however, cutaneous infection were the rule, I should expect to find all barefooted children in the infested area suffering not only from ground itch the entire summer, but also from severe infections of hookworm disease.

Hair.—The hair on the head appears to be about normal, but in cases contracted before puberty, the beard and the hair on the body (pubis, armpits, arms, legs) are usually undeveloped. I have seen patients 20 years of age upon whose body hairs were almost absolutely lacking.

Breasts.—The breasts of females, who have contracted hookworm disease before puberty, remain more or less undeveloped. In a girl of 20 years of age, for instance, the breasts may not be developed beyond those of a girl of 8 or 9 years old.

Nails.—The color of the tissue directly under the nails varies with the anemia.

HEAD.

Face.—The face has an anxious, stupid expression, and in severe cases is more or less "bloated" (edematous).

In fact, a prominent symptom of uncinariasis in practically all animals in which it occurs is the development, in severe cases, of a more or less extreme edema. To use the rural vernacular, "the face bloats," and "the feet and ankles swell." The symptom in question is more or less irregular in man as it is in other animals, notably in sheep, appearing and disappearing at intervals. Upon several occa-

sions I was informed that this symptom interfered seriously with the school attendance, for if the children sat still a long time in school "they began to swell." Quite generally, as was to be expected, testimony was to the effect that the edema was less frequent in the winter than in the summer.

Eyelids; conjunctivæ.—An examination of the eyelids exhibits the visibility of the blood vessels in light cases, but an absolute marble whiteness in very severe cases, with all possible intermediate stages corresponding to the general degree of anemia.

Eyes.—While looking at the eyelids for anemia, the observer frequently notices that the pupils are dilated or that they dilate readily and that the eyes are dull, dry, and usually of a chalky white. If the patient is directed to stare intently into the observer's eyes, there will be noticed a symptom which it is difficult to describe, but which I have found more constant than almost any other noticed, namely: After a moment, the length of time *apparently* varying slightly according to the degree of the disease, the pupils dilate and the patient's eyes assume a dull, blank, almost stupid, fish-like or cadaveric stare, very similar to that noticed in cases of extreme alcoholic intoxication. I am not familiar enough with the stare of anemic patients in general to state how common this peculiar look is among them, nor have I found any of my medical friends who could give me much information on this subject; but I can state that among the several scores of anemic people whom I examined on this trip, in the severe cases with two exceptions, I found the eggs of *Uncinaria americana* in every one (whose feces were examined) in whom I observed that indescribable stare; the two exceptions in question were city boys, both of them sons of a confirmed inebriate; further I failed to find the eggs present in certain extremely anemic patients in whom the stare was not noticed. It certainly was absolutely absent from a number of typical cases of malaria. Toward the end of the trip, I found myself unconsciously relying more upon the presence or absence of the blank stare than upon any other single symptom, except of course the presence of the eggs in the stools.

I will not go to the extent of stating that this stare is diagnostic for uncinariasis (and I will even warn that in dark eyes it is less evident than in eyes of light color), for I do not feel that I have had experience enough with the peculiarities of eyes in various diseases to speak authoritatively upon the subject. I simply mention this peculiarity in connection with the discussion of the eye as a symptom which, as my investigations progressed, made more and more of an impression upon me. Upon calling the attention of several local physicians to this peculiar stare, they informed me that it was a totally new symptom to them, but that after examining several cases they found it a very prominent symptom. As a general rule the eyes in advanced stages are dry. In this connection it may be noticed that several

authors have mentioned the glassy appearance of the eyes of dirt-eaters.

Since my return, several of my clinical friends with whom I have discussed this symptom have expressed some skepticism in regard to the matter. Although thoroughly convinced of its existence, for I saw it too frequently to be deceived, I have written to several Southern clinicians requesting them to give me the benefit of their independent observations on this point. Up to the time of reading "galley proof" of this report the following replies have been received:

[Extract from a letter from Dr. Hilsman, January 2, 1903.]

"Replying to your letter, 24th ultimo, I have to say that I have examined the patients that we saw in the country, and on making them gaze intently at me as you directed, I observed the vacant stare that you described, but did not observe the dilation of the pupils. The stare is very much like that of an epileptic as he begins to recover from a fit.

"These cases have improved under treatment suggested by you. The little girl passed a large number of the worms."

[Extract from a letter from Dr. M. A. Clark, January 30, 1903.]

"I have delayed, hoping to find the eyesymptom you mention, but I have not yet found it. My cases are improving slowly."

Dr. James Edward Stubbett, of New York, who spent some years in Central America and has seen many cases of hookworm disease, has stated to me that he has frequently observed the peculiar stare in the eyes of dirt-eaters.

In this connection it is also not uninteresting to note that some "worm doctors" claim to lay special stress upon the eye in making their diagnoses; also that dilation of the pupil, due to irritation by intestinal worms as well as to anemic conditions, is recognized by certain prominent writers on the eye; further, that dilation of the pupil is also a symptom upon which many children's nurses depend in suspecting the presence of worms.

Sandwith (1894, p. 12) states that the eyes of his patients showed a pearly white conjunctiva, singularly in contrast with the yellow color of the face. Dr. Scott examined several men for him with the ophthalmoscope, and found in half of them a normal fundus, and in the other half a very pallid fundus. The refraction tests showed astigmatism in many cases.

Nostrils.—The visible mucous membrane of the nostrils becomes pale in proportion to the anemia.

Lips and gums.—The lips also become pale in proportion to the anemia, the inner surface of the lips and the outer surface of the gums frequently presenting almost a chalky white appearance.

Teeth.—Irregularity of the teeth was so common among patients affected with uncinariasis that the question arose in my mind whether this was not to some extent an expression of the general underdevel-

opment of the body due to this disease. The point at issue calls for the opinion of a dentist rather than that of a zoologist. The decayed teeth of dirt-eaters have been recorded in early writings on this habit.

Tongue.—In some cases the tongue was coated. A number of observers have already called attention to this symptom, which was by no means general in the cases I observed.

Several authors have remarked upon the presence of black, brown, or purple spots on the tongue in cases of uncinariasis, and the view has been advanced that these present a valuable aid in diagnosis, and in some cases, at least, that they disappear on treatment. In the cases which came under my observation, I looked very carefully for this symptom. In many instances I found more or less distinct purplish to brownish spots, irregularly round or elongate in shape, and these may or may not be identical with the spots described by the authors cited. It was, however, noticeable that in many cases where these spots were observed, the patients were accustomed either to chewing tobacco or to dipping snuff. In fact, some of the spots in question I am inclined to refer to the use of tobacco or snuff.

Whether all cases are to be explained in this way is, however, open to question, with probabilities against such explanation. Nevertheless, it is well for practitioners to be forewarned upon this chance of error in diagnosis. Several authors have assumed that the spots on the tongue represent a symptom which has only recently been observed in this disease. In this connection, it is interesting to note that early authors—for instance, Cragin (1836a) and Imray (1843)—in writing upon dirt-eating have described these same spots.

NECK.

The cervical pulsations are often very evident, and in some cases may be seen from 6 to 12 feet away. (See Circulatory system, p. 72.)

THORAX.

In emaciation the thorax corresponds to the general emaciation of the extremities. In some cases the ribs are very prominent..

Heart.—See page 72.

Breasts.—See page 64.

ABDOMEN.

“Pot-belly” or “buttermilk-belly,” dropsy, and tympanites.—The condition known as “pot-belly” or “buttermilk-belly” is exceedingly common in uncinariasis, especially in extreme cases in summer. It is a distension of the abdomen, due apparently to two factors: (1) a gaseous distension of the bowels, and (2) the presence of an excess of fluid in the abdominal cavity.

I hasten to add that I have not seen a single autopsy in man infected with uncinariasis, but the same abdominal distention is found in

animals suffering from this disease, and a number of post-mortem examinations, especially on sheep, have given the conditions described.

Lutz mentions flatulence of the lower abdomen as a common symptom, and Sandwith (1894, p. 11) found it present to a slight extent in one-third of the cases.

Hair.—See page 64.

Genitalia.—See page 78.

EXTREMITIES.

Nothing of any particular note was observed in connection with the bones; for the muscles see p. 73. In many cases the feet and ankles were swollen, and in several cases the legs were marked with ulcers. See p. 59.

According to Sandwith (1894, p. 12), edema of the legs, like albuminuria, is to be seen only in the worst cases, and some of these had general edema when admitted in a dying condition.

GENITALIA.

See page 78.

MUCOUS MEMBRANES.

All mucous membranes correspond, in respect to their color, to the grade of anemia. (See Eyelids, p. 65; nostrils, p. 66; lips and gums, p. 66; tongue, p. 67; genitalia, p. 78.)

EXCRETIONS AND SECRETIONS.

URINE.

I have no observations to record in regard to the urine. Sandwith (1894, p. 11) states that—

“The urine is not unlike that of ordinary anemia, neutral or alkaline in equal proportion, and rarely acid, pale colored, with specific gravity ranging from 1010 to 1015. A trace of albumen was present in all the most advanced cases, without casts under the microscope.”

According to Zinn and Jacoby (1898, p. 16), Lussana (1890) found in the urine of patients suffering from uncinariasis a poisonous substance of the nature of a ptomaine, which caused extensive changes in the rabbit's blood, especially a change in the number of red blood corpuscles, and also poikilocytosis. These results are said to have been confirmed by Arslan (1892).

FECES.

Consistency.—The feces may be hard or soft, according to the presence of constipation or diarrhea.

Reaction.—In reaction the feces may be acid, alkaline, or neutral.

Color.—In a large proportion of medium and severe cases, the feces are reddish to brown in color. In some cases blood is present.

Blotting paper test.—In about 8 out of 10 medium and severe cases, if a portion of the feces is placed upon white blotting paper, and allowed to remain there for twenty minutes to several hours, it leaves on the paper a reddish brown stain similar to a blood stain. This test will be found useful by physicians who are not prepared for microscopic tests.

Microscopic examination.—If feces less than twenty-four hours old are examined microscopically, the eggs will be found in various stages of segmentation. If feces over twenty-four hours old are examined the free embryos also are usually found. If free embryos are found in perfectly fresh feces, the diagnosis of infection with the worm (*Strongyloides stercoralis*^a) of Cochin-China diarrhea must be made, and this parasite we may find in the same patient in whom uncinariasis is present.

CIRCULATORY SYSTEM.

The symptoms of the circulatory system are the most marked and most common; they seem to develop after the symptoms of the digestive system, but before those of the nervous system.

ANEMIA.

In all medium and severe cases, the anemia is what first attracts attention. This varies in intensity not only in proportion to the degree of infection, but also to a considerable extent in proportion to the length of the period of infection. For instance, 100 worms may be expected to produce a greater anemia within a given time, say one year, than will 50 parasites. But 50 worms may be expected to produce more anemia in two years than in one.

In some medium cases, the blood vessels of the conjunctivæ may be more or less visible, and the visible mucous membranes of the nose, mouth, and vulva, may be more or less of a normal or subnormal color. In the extreme cases, these structures may be as white as marble or paper. In the same way the color of the skin will vary (see Skin, p. 59; Nails, p. 64) from an almost natural hue to a whitish, lemon yellow, or tan color.

^aThis worm is probably much more common in this country than supposed. The cases known to me to date are: Baltimore, Md.—1 case, 1 death; reported by Strong, 1901. Richmond, Va.—1 case, 0 death; reported by Thayer, 1901. Anne Arundel County, Md.—1 case, 0 death; reported by Thayer, 1901. Washington, D. C.—4 cases, 0 death; unpublished, Zool. Lab., U. S. Public Health and Marine-Hospital Service. Ohio—1 case, 0 death; unpublished, Dr. A. P. Ohlmacher (mentioned here by kind permission of the observer). San Francisco, Cal.—3 cases, ? deaths; unpublished, Dr. P. K. Brown (mentioned here by kind permission of the observer). Cuba—? cases, ? deaths; unpublished, Dr. John Guiteras (mentioned here by kind permission of the observer). Porto Rico—? cases; ? deaths; unpublished, Dr. P. K. Brown.

Blood.—I did not stop for blood counts, as these have been made by other men, and while they are exceedingly interesting from a pathological standpoint they have not appealed to me as so direct a method of diagnosing intestinal parasites as is the fecal examination. In general it may be said that the blood of man corresponds to the blood of sheep, goats, cattle, dogs, etc., suffering from the same disease; in other words, the severer and longer the infection, the thinner the blood. In the vernacular of the sandlapper, it is "like water." It may here be added that in early literature on dirt-eating, several authors remark upon the water-like appearance of the blood.

Speaking in more technical language, the blood has been shown by Ashford to possess the following characteristics:

"(1) A severe anemia, falling as low as that of Addison's anemia in count of red cells in some cases. (2) A very low hemoglobin average and a very low color index. (3) A marked eosinophilia in some cases; 40 per cent reached in one case. This follows the observation of Neusser. (4) No leucocytes common to the disease itself. Leucocytosis recorded is always apparently due to complications, as noted. (5) Frequent presence of normoblasts, and in some cases megaloblasts, but never a majority of megaloblasts. (6) Poikilocytosis common. Manson denies this."

Ashford gives the following interesting table of blood counts:

Schedule of blood examinations.

Date.	Current number.	Red cells.	Hæmoglobin.	White cells.	Percentage of polymorphonuclear leucocytes.	Percentage of small lymphocytes.	Percentage of large lymphocytes.	Percentage of eosinophiles.	Normoblasts per cubic millimeter.	Megakaryoblasts per cubic millimeter.	Polkilocytosis, Digitalis and peptomangan.
November 4 ...	1	1,530,116	20	6,800	65	21	9	5	26	13	Polkilocytosis. Digitalis and peptomangan. Recount, November 23, 1899; Hæmoglobin, 10 per cent; reds, 1,800,000.
November 3 ...	2	697,776	20	7,960	59	17	15	9	144	46	Polkilocytosis. Polychromatophilia, many macrocytes and microcytes. Treatment: Pili ferri, arsenic, et strychni, and pili arsenici. Recount, November 23, 1899; Hæmoglobin, 25 per cent; reds, 2,664,440.
November 4 ...	3	1,533,112	22	2,000	64	22	8	6	8	0	Polkilocytosis. No rouleaux. Treatment: Digitalis and peptomangan. Recount, November 23, 1899; Hæmoglobin, 23 per cent; reds, 1,973,328.
November 5 ...	4	1,200,000	15	4,200	64	23.4	6	6.6	109	8	Polkilocytosis. Polychromatophilia. Treatment: Digitalis, stryp, ferri iodidi. Recount, November 23, 1899; Hæmoglobin, 17.5 per cent; reds, 801,104.
November 6 ...	5	1,484,440	10	6,000	64	24	8	4	12	0	Polkilocytosis. Slight tendency to rouleaux formation; macrocytes and microcytes, latter predominate over former. Recount, November 23, 1899; Hæmoglobin, 14 per cent; reds, 687,776.
Do.....	6	2,193,328	23	8,800	65	26	8	14	123	35	Polkilocytosis. No tendency to rouleaux formation. Treatment: Digitalis, pili ferri, quin, et strychni.
November 7 ...	7	1,633,328	17	5,600	60	23	5	12	11	0	Polkilocytosis. Many macrocytes and microcytes. Slight rouleaux formation; marked chromotophilia. Treatment: Digitalis, liq. potas arsenitis in increasing doses to grt. 5 i. l. d.
Do.....	8	2,064,664	23	4,800	74	17	6	8	28	0	Polkilocytosis. Rouleaux formation good. Treatment: Pili ferri, quin, et strychni, and digitalis. Recount, November 23, 1899; Hæmoglobin, 31 per cent; reds, 3,084,440.
Do.....	9	1,271,104	14	7,800	60	17	6	17	30	15	Has elephantiasis arabum, but I can not identify the filaria as yet. Microcytes predominate over macrocytes. Good rouleaux formation. Treatment: Peptomangan. Recount, November 23, 1899; Hæmoglobin, 24 per cent; reds, 2,520,000.
Do.....	10	1,800,000	17	1,500	72	20	4	4	6	0	Polkilocytosis. Recount, November 23, 1899; Hæmoglobin, 13 per cent; reds, 668,888.
November 8 ...	11	1,800,000	25	4,600	69	22	7	2	9	0	Polkilocytosis. Rouleaux formation good.
Do.....	12	2,296,656	30	7,680	62	26	12	10	0	0	Polkilocytosis. Rouleaux formation slight; has abscess of the liver.
November 9 ...	13	1,268,888	20	6,800	63	17	7	13	0	0	Polkilocytosis. Rouleaux.
Do.....	14	2,440,000	25	11,000	50	10	9	31	0	0	Tertian malarial parasites found. Rouleaux good.
Do.....	15	2,353,328	17	12,700	73	17	4	6	10	10	Polkilocytosis. Rouleaux good. I believe this man to have been suffering from pneumonia at the time of examination, although the differential seems strange.
Do.....	16	2,634,444	23	5,200	72	20	5	3	38	0	Only case not presenting ankylostomes. Has tuberculosis.
November 10 ...	17	2,140,000	23	18,000	40	12	8	40	0	0	Polkilocytosis. Rouleaux formation good.
November 20 ...	18	3,524,440	30	14,800	88	9	3	1.5	0	0	
November 12 ...	19	(C)	30	9,000	60	16	10	10	125	
November 20 ...	20	1,560,000	16	2,400	72	10	5	13	0	0	

α Unknown.

Cervical pulsations.—In the rural vernacular, “jerking at the neck” or “jumping at the neck” refers to an anemic symptom which is exceedingly prominent in most medium cases and in all extreme cases of uncinariasis. It is simply the violent pulsations of the cervical vessels, visible sometimes at a distance of 2 to 4 meters.

Heart.—Nearly all medium and severe cases complain of having “heart disease” or a “fluttering of the heart,” and many of the patients are taking medicine for this symptom. (See also p. 35.) We have here, of course, the usual cardiac symptoms of an extreme anemia.

“Palpitation over the heart, in the epigastrium, and in the temporal arteries is sure to be present in bad cases, while the anemic murmurs of heart and neck are solely dependent upon the degree of anemia, and can be banished by a prolonged course of iron. Hypertrophy of heart was noted and verified after death in some of the advanced cases.” (Sandwith, 1894, p. 12.)

Pulse.—Pulse varies from 80 to 132 per minute. In medium and severe cases I noticed about 120 per minute probably more frequently than either a higher or a lower pulse. This was found in young and middle-aged (probably more commonly in children), in males and females, and yet without a temperature which was distinguishable by the hand as especially high.

TEMPERATURE.

Not being able to follow any cases for any length of time, hence not being able to make continued observations on the temperature, I considered that observations in other lines were more important under the circumstances. Hence I did not take temperatures carefully. According to observations by various clinicians, there may be subnormal or normal temperature, or the thermometer may register 100° to 102° F.

“The skin is always cold, and the temperature before thymol generally subnormal in uncomplicated cases. After excluding any fever produced by concurrent diseases and any defervescence caused by thymol, I find that one-third of my patients had a normal temperature during their stay in the hospital, but that two-thirds had a distinctly subnormal range, varying from an average of 36.3° C. a. m. to 36.9° C. p. m.

“Many of these patients when convalescent had an increase of half a degree, night and morning.

“Surgeon-Major Giles suspected that many of his patients in Assam had suffered from fever at the onset of their malady, and he was confirmed in this impression by observing pyrexia in the monkeys he fed on *anchylostoma* embryos. After eliminating all those who had fever in the hospital, or a history of intermittent fever or any enlargement of the spleen, I found that 68 per cent of the remaining stated that their trouble had begun with a few days’ fever.” (Sandwith, 1894, p. 12.)

RESPIRATORY SYSTEM.

NOSTRILS.

See page 66.

RESPIRATION.

Many patients complain of a difficulty in breathing, especially after exertion. This symptom is quite natural, in view of the low condi-

tion of the blood and the emaciation of the muscles. Respiration is rather variable and does not appear to be a symptom of very great value; it may be slow, or it may be increased to about 30 or more per minute. According to Sandwith (1894, p. 12) dyspnea and noises in the ears were present, as might be expected with marked anemia.

MUSCULAR SYSTEM.

EMACIATION.

A progressive emaciation is more or less common, especially in severe cases. The arms and legs seem to be reduced to skin and bones; the chest is so emaciated that the ribs are very prominent and the beating of the heart is very evident. What little muscle is left is soft and flabby.

Emaciation is, however, not present in all cases, and even in some medium infections the muscles may be well formed and more or less hard. I recall one case in particular: A boy about 14 who showed a heavy infection microscopically, a clear clinical history of uncinariasis of several years standing, decided anemia, distinct cervical pulsations, abdomen rather distended ("pot-bellied"), yet his arms and legs were well formed and his muscles surprisingly solid for a patient in his condition.

Sandwith (1894, p. 13) found the average weight of 100 grown men upon admission to the hospital to be 117.5 pounds; the average height of these men was 5 feet 5.5 inches, which by Dawson's tables should scale at least 135 points. Of the patients who stayed in the hospital more than two weeks 70 per cent gained weight, 22 per cent lost, and 8 per cent remained stationary. The average loss of weight was 3.2 pounds, and was, of course caused by the necessary starvation, thymol, and purging. The average gain was 5.4 pounds, some patients gaining as much as 15, 17, 18, or 20 pounds.

GREAT PHYSICAL WEAKNESS.

One of the most pronounced symptoms complained of is a general weakness. The patient states that he is obliged to rest after exertion. In light cases a feeling of lassitude is experienced without being able to assign it to any particular cause; as a result, it is generally assigned by other people to laziness. In medium cases the patient may be able to work one to three or four hours before becoming exhausted; in very severe cases he will scarcely be able to walk across the room, or he may be confined to the bed for weeks at a time. A physical examination usually shows an emaciation proportionate to the weakness.

DIGESTIVE SYSTEM.

LIPS, GUMS, TEETH, AND TONGUE.

See p. 66.

NAUSEA.

Nausea was not noticed. Sandwith (1894, p. 11) states that vomiting and nausea are rarely complained of.

APPETITE.

The appetite may be light or ravenous. According to Sandwith, the appetite is invariably affected, sometimes ravenous at beginning, but later always capricious and diminished. The English nurses report to him that the patients were always begging for medical comforts or cigarettes, even in the middle of the night, when other patients were asleep. Among 40 men carefully examined, 16 said that their appetite was once greatly exaggerated, 16 pleaded diminution from the beginning of their illness, and 8 believed that their appetite was normal until the anemia became very marked.

Perverved appetite, "Dirt-eating."—The most important point to be noticed in connection with the appetite is the abnormal desire for some particular article of food. Frequently this is a preference for something sour or bitter.

Many patients with uncinariasis are known throughout the village or county as being especially fond of pickles. I have seen boys and girls in advanced cases of this disease who would greedily devour an entire bottle of pickles. Some patients are especially fond of sucking lemons, or lemons and salt, or salt alone. Others are known for their desire to chew coffee, or to drink large quantities of strong coffee without milk or sugar. Some are abnormally fond of buttermilk. Others are noted as "resin-chewers." Some are accused of "lapping sand." Many are accused of eating clay or dirt.

Dirt-eating has been discussed by a number of authors, opinion being divided as to its status. Some writers look upon it as the cause of the disease; others view in the habit only a symptom or a result; still others consider it nature's treatment of a diseased condition.

Among helminthologists there seems to be the impression that dirt-eating is especially likely to lead to infection with parasites. Among Southern physicians I found the idea quite prevalent that dirt-eating was one of the causes of the condition which I have classed as extreme uncinariasis.

During the trip now under discussion, I have had opportunity to observe many so-called dirt-eaters. As most authors state, it is exceptional that one will acknowledge that he eats dirt. I believe the explanation of this denial is very clear, namely, not only is there a certain amount of disgrace connected with the reputation of being a dirt-eater, but probably not over one person in ten, or possibly

in twenty, accused of eating dirt ever does so. The other nine to nineteen have their abnormal appetites developed in a different direction, namely, pickle-eating, lemon-sucking, coffee-chewing, resin-chewing, etc.

Among the articles eaten by these "dirt-eaters," various authors mention charcoal, chalk, dried mortar, mud, clay sand, gravel, stones, shells, rotten wood, cloth, garments, paper, tobacco pipes, mice, young rats, etc.

It is, I believe, an error to attempt to reduce this abnormal habit to any one common basis. In general, however, it may be stated that the alleged "dirt-eating" in this country practically represents the severe cases of uncinariasis. To attempt to reduce dirt-eating to infection with worms, particularly with *Uncinaria americana*, will doubtless be thought extreme, more particularly by Northern physicians. Still the idea is not a new one, and a moment's consideration will show that this view is far less extreme than it at first appears.

For an excellent general discussion of dirt-eating, with extensive references to literature, the reader is referred to Le Conte (1845). For the purpose of the present paper it will suffice to call attention to certain facts and analogies. The habit of eating slate pencils, paper, and other objects by chlorotic girls is more or less commonly known. Pregnant women, also, may develop an abnormal appetite, which takes different phases, including dirt-eating. It is recorded that the Javanese women eat certain dirt in order to improve their appearance. In certain localities in tropical America (Orinoco) the natives eat earth during the overflow of the river when they can not obtain their regular food. Earth eating is said to be common and not injurious in certain parts of Africa. According to Sandwith (1894, p. 9), on the day of the maximum high Nile, and the general rejoicings thereupon, the town crier, who is on the lookout for backsheesh, presents "teen ibliz" (Nile mud) with a lemon to the inhabitants for luck, and many of them eat of it. Dogs, horses, cattle, hogs, and alligators are recorded as eating clay and sticks. The Alaskan seals, when infected with round worms, eat pebbles. Elephants, when infected with flukes, eat a certain kind of clay until a looseness of the bowels is produced. I have frequently heard Texas grangers attribute the death of cattle to eating sand, and in post-mortem examinations of cattle, sheep, and goats, in an anemic condition from intestinal worms (verminous gastritis caused by *Hæmonchus contortus*, *H. Ostertagi*, etc., and infection of small intestine with *Uncinaria trigenocephala* and *U. radiata*), I have repeatedly noticed in the stomach and intestine large quantities of sand; so that the farmers present declared that this was the cause of death. Dogs infected with intestinal worms eat grass. Cats also frequently eat grass, probably from the same cause. Children infected with eel worms (*Ascaris lumbricoidea*) are known

occasionally eat dirt, and I know of one such case where the habit ceased when the worms were expelled.

In view of the comparisons cited, it would seem that the idea of considering dirt-eating as a manner of infection with parasitic worms, although conceivable for some cases, is hardly correct as applied to most instances. That dirt-eating is an abnormal appetite due to a diseased condition (anemia and a disordered digestive system) as suggested by several authors as early as the first half of last century, seems to me to be an explanation of much more general application; and that this anemia and enteritis or gastritis may be produced by parasitic worms is an established fact. In this connection, it is interesting to note that Hancock (1831, p. 67), in discussing dirt-eating, mentions "worms preventing the nourishing effects of food;" Imray (1843, p. 310) remarks that "worms in considerable numbers were not uncommonly accumulated in the intestinal canal." Further, it is significant that various authors, in discussing the treatment of dirt-eating, attribute more or less success to certain drugs which are in fact used more or less in treating for intestinal parasites. Thus, Cotting (1836a) refers, as stated above, to the decrease of dropsy and of dirt-eating corresponding to the more general use of calomel; sulphate of iron is mentioned by Cragin (1836a), Pollard (1852), and others; according to Pollard (1852), copperas is a popular and successful remedy among the negroes; Hancock (1831) refers to a remedy containing arsenic as having had great success; Jordan (1832) states that dirt-eating decreased upon destroying the huts and moving the families to some other location.

To summarize: While it would seem decidedly extreme and unwarranted to maintain that dirt-eating is necessarily an indication of infection with intestinal worms, still I believe the conclusion is justified that it is undoubtedly a more or less common tendency in such infections, not only in man but also in other animals. It may be classed with the chewing of slate pencils, resin, coffee, sucking of lemons and salt, etc., as an abnormal appetite due to the anemia and abnormal condition of the intestinal tract. Further, for all practical purposes it is not much of an exaggeration to look upon most, if not all, so-called dirt-eaters of the sand areas of our Southern States as representing severe cases of uncinariasis.

Sandwith (1894) states that 26 per cent of his patients confessed to eating earth, and he refers to "earth hunger" as sometimes the cause and sometimes the effect of hookworm disease.

PAIN IN THE STOMACH; INDIGESTION.

Many patients complain of colicky pains "in the stomach," and will indicate the region between the navel and the ensiform cartilage as the seat of the "misery." Indigestion is frequently mentioned, and the tongue is occasionally coated.

Just how much the indigestion is due to uncinariasis and how much to other causes may be considered an open question. Foul breath is mentioned by some authors as a common symptom of uncinariasis, but this has not been particularly noticeable in many of the cases I saw.

Many authors explain the tendency to dirt-eating as an effort to neutralize the hyperacidity of the stomach. As I have just shown (p. 74), however, many patients with uncinariasis eat pickles and suck lemons.

According to Sandwith, a gnawing, throbbing pain in the epigastrium is the first symptom complained of, chiefly because it is constant, whereas a severe colic and borborygmi (rumbling of bowels caused by gas) of intestine are present from time to time. I was unable to confirm the constancy of the pain.

CONSTIPATION AND DIARRHEA.

Sandwith (1894, p. 11) states that when the patient is not under thymol and purgative treatment, constipation is a very constant symptom in hospital cases; 60 per cent had suffered for a long time from obstinate constipation, 28 per cent had had diarrhea before admission, and 12 per cent had no recollection of being troubled with either. "None of the figures depending upon the memory of the patients must be taken as absolute truth, as the intelligence of many is of a very low order." Diarrhea, and even dysentery, are not uncommon in very advanced cases, especially those complicated with *Bilharzia* (= *Schistosoma*, which has not been reported as endemic in the United States), or ulceration in the rectum; and unless the patient is robust enough to support thymol, such cases are apt to end fatally.

In my own cases I found both constipation and diarrhea, but I am not in a position to state that either symptom was regular or characteristic for any given degree or stage of infection. In severe cases diarrhea was certainly more or less common.

Feces.—See page 68.

NERVOUS SYSTEM.

The nervous symptoms usually develop later than either the intestinal or the circulatory symptoms.

EYES.

See page 65.

EARS.

According to Sandwith (1894, p. 12), noises in the ear are present. None of my patients complained of this symptom.

MENTAL LASSITUDE, HEADACHE, DIZZINESS, AND NERVOUSNESS.

Not only does physical exertion result in exhaustion, but mental exertion has to be avoided. The children complain that they are unable to study and that any continued application to books results in

severe headache. This feature of the disease is fully confirmed by the testimony of both teachers and parents, who assert that children of this class are usually much more backward (and even stupid in their studies) than other children not showing the symptoms under discussion.

Dizziness is very commonly mentioned by the patients. This feeling, which they usually speak of as a "swimming in the head," is experienced especially upon rising suddenly from a chair or a bed.

Nervousness does not seem to be so commonly complained of as mental lassitude, headache, and dizziness. Still it is more or less frequently mentioned, more particularly by the girls and women.

Among girls from about 13 to 20 years of age it was quite noticeable that they were more timid and more emotional than were their healthier sisters.

According to Sandwith (1894, pp. 11-12), there is pain in the head, generally referred to the temples, while in the knees there is almost invariably present great weakness and some pain; occasionally there is in addition pain in the shoulders. Giddiness is another very general symptom, and it is this as much as anything else which compels the patients to give up work. The worst cases are those which are nearly always asleep, and can not be interested in anything when they are awake. On the whole, sleepiness is decidedly a symptom. Dense stupidity, associated sometimes with reiterated demands for a favor already granted, shows that the bloodless brain is affected in all advanced cases, and at least three times Sandwith (1894, p. 13) found a condition of weak-mindedness which would have warranted the patients being sent to the asylum.

PATELLAR REFLEX.

Absence of patellar reflex is reported in cases of general debility and muscular weakness. Sandwith (1894, p. 13) found this reflex unaltered in 35 per cent of the cases examined, completely absent in 48 per cent, decidedly diminished in 5 per cent, and a little exaggerated in 12 per cent, all of which were early cases.

GENITAL SYSTEM.

EXTERNAL GENITALIA; SEXUAL FUNCTION; MENSTRUATION; STERILITY.

In cases where infection has taken place in early childhood, the delayed development of the genital system is very marked. Patients of 16 to 22 years of age may not be better developed than healthy persons of 11 to 15 years. Menstruation may be very irregular, especially in summer. This same condition is insisted upon in many early writings on dirt-eating. Mothers frequently ascribe the condition of their daughters to the absence or irregularity of the menstruation as is mentioned also in early writings on dirt-eating.

Sandwith (1894, p. 13) found impotence to be a decided symptom in hookworm disease. Of 38 men especially examined on this point, 24 had completely and 5 had almost entirely lost their virile power, while of the remaining 9 men, 5 aged from 19 to 25 had their puberty considerably delayed.

TENDENCY TO ABORTION.

Among women affected with uncinariasis I found a marked tendency to abortion. Given a woman about 28 years old who had been married nine years—a not uncommon history is that she has had 3 to 5 children and 3 to 4 miscarriages, and she looks to be about 50 years old. Not being able to follow these cases through their entire medical history and the history of their husbands, I must leave the question open as to how many of these abortions are to be attributed to uncinariasis and how many are due to other causes. In the country districts I was thrown in with the anemic not with the healthy families, hence I have no good basis for comparison of these two classes for the particular localities visited. In some cases a history of venereal disease was suspected or admitted; in others, the abortion came on after pitching fodder; in some cases the patients had taken more or less quinine during their life, under the supposition that they had malaria; and in still other cases, my suspicions were aroused in other directions. The determination of the exact relation of uncinariasis to the miscarriages, which are certainly strikingly prevalent, must be left to those who can follow the cases for a longer period of time.

PREVALENCE OF UNCINARIASIS IN THE UNITED STATES.

In several earlier papers (1901, p. 524; 1902 a, p. 778; 1902 b, pp. 183, 212) I have advanced the view that uncinariasis must be more common in this country than is generally supposed. In my preliminary report on this trip (see above, p. 35), I said that: "There is in fact not the slightest room for doubt that uncinariasis is one of the most important and most common diseases of this part [South Carolina] of the South, especially on farms and plantations in sandy districts."

Harris (see above, p. 36) went even farther than this and claimed that uncinariasis is "the most common of the severe diseases of the South."

In considering the subject of the frequency and economic importance of the disease under discussion, I do not wish to seem to underestimate the prevalence of tuberculosis and of venereal diseases among the negroes or of malaria among the whites. Further, I recognize the fact that at the present moment an exact mathematical estimate can not be made. Speaking in general terms, however, the facts at my disposal at present seem to indicate that taking the Southern Atlantic

States as a whole, uncinariasis must be considered as one of the most common and widespread maladies; in frequency it belongs in the general class with malaria, tuberculosis, and gonorrhea.

In cities and in rural clay districts it is probably less common than any one of these three maladies, for such localities may present local foci of infection for the diseases in question, while the local foci of infection with uncinariasis are much more limited.

Among the negroes of the rural sand districts, uncinariasis seems to be much less common than either tuberculosis or gonorrhea. Its apparent rarity may, however, be deceptive (see p. 51).

Among the whites of the rural sand districts, uncinariasis is apparently the most common disease found. Nevertheless, in some sand districts, probably with a clay or other impervious subsoil favorable to the formation of marshes, malaria rivals uncinariasis for first place.

From these qualified statements it will be seen that I do not feel justified in adopting the view advanced by Harris, namely, that uncinariasis is "the most common of the severe diseases of the South."

In all probability, further study will show that in Mexico, Central America, and parts of South America, hookworm disease is more important and more common than in the United States.

Sandwith (1894, pp. 5-6), in discussing the frequency of this disease in Egypt, says:

"It is impossible to know what amount of the population [of Egypt] is affected, but the statistics of the recruiting commissioners for 1892 are worth quoting. Nearly every adult male peasant is liable for conscription, and the conscripts are immediately examined in their villages. In upper Egypt 5,988 men were called, and 200, or 3.3 per cent, were rejected for anæmia. In lower Egypt 661, or 6.2 per cent were rejected from this cause out of 7,420 men. Every province furnished anemic rejections, but Menoufieh came highest on the list with 13.9 per cent, while I find from hospital statistics that no less than 15 villages in that province are infected. The recruiting medical officer, who is an Englishman, only rejects those who are obviously too anæmic to serve with the colors, accepting many who are already the hosts of the bloodsucking worm. Thus the medical reports for the Egyptian army show that in 1890 there were 114 admissions to the hospital for anæmia, in 1891, 107 admissions, and in 1892, 170 admissions. In 1891, 22 soldiers were invalided from the service for anæmia, and 65 in 1892, besides 1 death. The number of admissions for debility is equal to those for anæmia, and doubtless includes many cases of anchyllostomiasis."

Dobson (1893, p. 63), examined 547 of the healthiest looking coolies from India and found hookworms in no less than 454 of them.

CLINICAL DIAGNOSIS OF HOOKWORM DISEASE.

As stated above, a man who is familiar with this disease should have no difficulty in recognizing severe cases, especially if he is in the area of infection. In light and medium cases, however, it is unsafe to make a diagnosis upon symptoms alone, unless such cases are associated

in the same family or neighborhood with severe cases. The best and most reliable method of diagnosis is by fecal examination, although in blood examination increased eosinophilia indicates the possibility of intestinal parasites.

First of all let us recall that uncinariasis is a possibility which should be considered in connection with all cases of anemia, especially among earthworkers, as in miners, brickmakers, canal diggers, farmers, etc., or in persons returning from the tropics, and among persons who have a history of residence on sandy soil. Three methods of fecal examination are open to us—the blotting-paper test and the microscopic and the gross examinations.

BLOTTING PAPER TEST WITH FECES.

For persons who are not in a position to make a microscopic examination, the blotting-paper test (referred to on p. 69), will be found very useful. To make the test, use only fresh feces. Place an ounce or more of the stool on a piece of white blotting paper (any absorbent white paper will answer the purpose); allow it to stand for twenty to sixty minutes; remove the feces and examine the color of the stain. In about four out of five cases of medium or severe uncinariasis, the stain is reddish brown and immediately reminds one of a blood stain. In making this test on anemic patients, piles should of course be excluded.

It developed in my work in the Virginia penitentiary, that this test is open to error in dealing with criminals. In order to avoid work, convicts, especially hard-labor contract convicts, occasionally produce a hemorrhage purposely by wounding the mucosa of the rectum by means of some sharp instrument.

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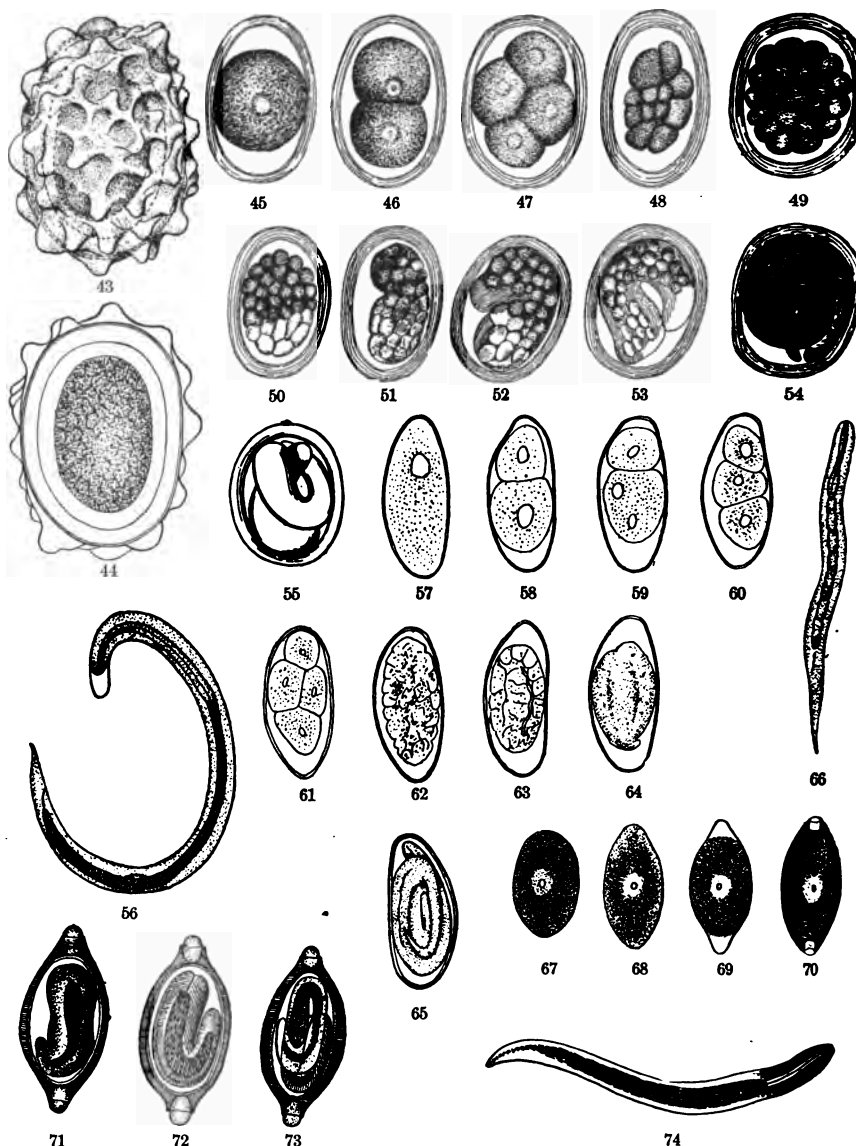


FIG. 43.—Egg of the common ascaris (*Ascaris lumbricoides*) of man, as found in feces. Seen with superficial focus. Greatly enlarged. (After Stiles, 1902b, p. 202, fig. 158.)
 FIG. 44.—The same, as seen with median focus. Greatly enlarged. (After Stiles, 1902b, p. 202, fig. 159.)
 FIGS. 45-54.—Embryology of the common ascaris (*Ascaris lumbricoides*) of man, showing the changes undergone by the egg after being discharged in the feces. (After Leuckart, 1867, p. 213, fig. 154.)
 FIG. 55.—Embryo of the common ascaris (*Ascaris lumbricoides*) of man, in the eggshell. (After Leuckart, 1867, p. 215, fig. 156.)
 FIG. 56.—Free embryo of the common ascaris (*Ascaris lumbricoides*) of man, casting its skin. (After Leuckart, 1867, p. 214, fig. 155.)
 FIGS. 57-64.—Embryology of the common pinworm (*Oxyuris vermicularis*) of man, showing the changes undergone by the egg while in the female worm. (After Leuckart, 1868, p. 322, fig. 191.)
 FIG. 65.—Embryo of the common pinworm (*Oxyuris vermicularis*) of man, in the eggshell, as found in fresh feces. (After Leuckart, 1868, p. 328, fig. 196.)
 FIG. 66.—Full-grown embryo of the common pinworm (*Oxyuris vermicularis*) of man, after it has escaped from the eggshell. (After Leuckart, 1868, p. 328, fig. 195.)
 FIGS. 67-70.—Egg of the common whipworm (*Trichuris trichiura*) of man, showing changes undergone while still in the female worm; fig. 70 is the stage found in fresh feces. (After Leuckart, 1868, p. 491, fig. 275.)
 FIGS. 71-73.—Later stages of development of an allied whipworm (*Trichuris affinis*) of sheep and cattle, showing changes after the egg escapes in the feces. (After Leuckart, 1868, p. 494, fig. 276.)
 FIG. 74.—Isolated embryo of *Trichuris affinis*. (After Leuckart, 1868, p. 495, fig. 277.)

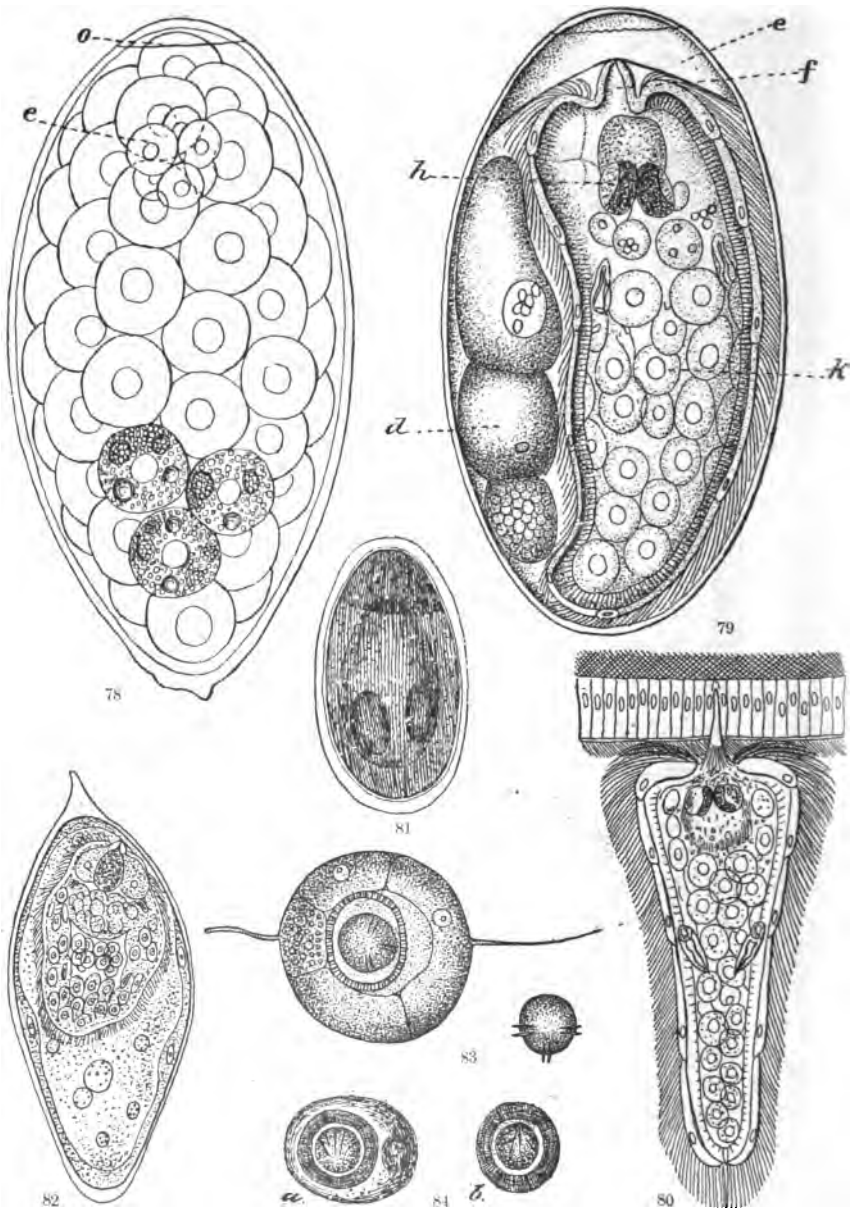


FIG. 75.—Egg of Cochin-China diarrhea worm (*Strongyloides stercoralis*) found in stools. (After Thayer, 1901, pl. 9, fig. A.)

FIG. 76.—Rhabditiiform embryo of same, from the stools. (After Thayer, 1901, pl. 9, fig. B.)

FIG. 77.—Filariform larva of same derived, by direct transformation, from a rhabditiiform embryo. (After Thayer, 1901, pl. 9, fig. C.)

Figures 75 to 77 were drawn from life, as seen under Leitz, objective 7—ocular 8.



- FIG. 78.—Egg of the common liver fluke (*Fasciola hepatica*) examined shortly after it was taken from the liver of a sheep; this is the same stage that is found in human feces; at one end is seen the lid or operculum, *o*; near it is the segmenting ovum; the rest of the space is occupied by yolk cells which serve as food; all are granular, but only three are thus drawn. X 680. (After Thomas, 1883, p. 261, fig. 1.)
- FIG. 79.—Egg of the common liver fluke containing a ciliated embryo (miracidium) ready to hatch out; *d*, remains of food; *e*, cushion of jelly-like substance; *f*, boring papilla; *h*, eye-spots; *k*, germinal cells. X 680. (After Thomas, 1883, p. 283, fig. 2.)
- FIG. 80.—Embryo of the common liver fluke (*Fasciola hepatica*) boring into a snail. X 370. (After Thomas, 1883, p. 285, fig. 4.)
- FIG. 81.—Egg of lancet fluke (*Dicrocoelium lanceatum*) with contained embryo. X 700. (After Leuckart, 1889, p. 379, fig. 171.)
- FIG. 82.—Egg of human blood fluke (*Schistosoma haematobium*) with contained embryo, passed in the urine or in the feces. X 285. (After Looss, 1896, pl. 11, fig. 112.)
- FIG. 83.—Egg of beef-measure tapeworm (*Tenia saginata*) with thick eggshell (embryophore), containing the six-hooked embryo (oncosphere) enlarged. (After Leuckart.)
- FIG. 84.—Eggs of pork-measure tapeworm (*Tenia solium*): *a*, with primitive vitelline membrane; *b*, without primitive vitelline membrane, but with striated embryophore. X 450. (After Leuckart, 1880, p. 667, fig. 297.)

No special technique is necessary. Simply take a small amount of feces, preferably from near the surface, about the size of the head of a large pin; spread this out in a drop of water on an ordinary microscopic slide and cover the preparation with a cover slip. Examine under any moderately high power, as a Zeiss 8 mm., Zeiss C, or a Bausch & Lomb one-third inch. Look carefully, with not too strong illumination, for an elongate oval egg with thin shell, and with protoplasm either unsegmented or in the early stages of segmentation. The older the feces and the warmer the weather the more advanced will be the segmentation. In case of infection with *Uncinaria americana* the fully developed embryo may be found within the eggshell. Be cautious not to mistake for the egg of the *Uncinaria* the eggs of *Ascaris lumbricoides*, which have a thick gelatinous, often mammillated, covering and an unsegmented protoplasm (figs. 43-44), or the eggs (figs. 57-65, of *Oxyuris vermicularis*, with a thin asymmetrical shell (one side being almost straight) and containing an embryo, or the eggs of whipworms (*Trichuris trichiura*, more commonly known to physicians as *Trichocephalus dispar*), possessing a smooth, thick shell, apparently perforated at each pole, and an unsegmented protoplasm (fig. 70).

As a rule, in fecal examination I prefer to use the thick, large, 2 by 3 inch slide, such as is used in examining for trichinæ, rather than the ordinary 1 by 3 thin "English slide." The larger slide is not only more steadily and more easily manipulated in case one is working without a mechanical stage, but it is much cleaner to handle.

In most cases of infection with intestinal worms the simple method just described will suffice for a positive diagnosis. Before giving a negative opinion, however, I invariably make ten preparations or follow a procedure which we may call "sedimenting the feces." Experience has shown me that in cases of negative diagnosis by the simple method positive diagnosis occasionally results if the feces are washed and "sedimented."

Method of washing and sedimenting feces.—Take one or two ounces of feces, fresh or dry, mix with water, and place in a large bottle, retort, jar, or any other receptacle; add enough water to make from a pint to two quarts, according to the amount of feces; shake or stir thoroughly and allow to settle; pour off the floating matter and the water down to near the sediment; repeat the washing and settling several times, or as long as any matter will float. The last time this is done use a bottle or graduate with a smaller diameter, and when the material is thoroughly settled examine the fine sediment. It will be

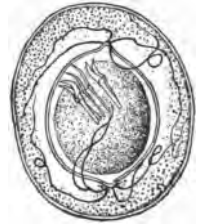


FIG. 85.—Egg of the dwarf tapeworm (*Hymenolepis nana*) of man. Greatly magnified. (After B. H. Ransom, unpublished.)

found that the eggs have settled more numerous in the fine sediment than in the coarse material.

In case an unusual amount of large coarse material is present in the feces, it is sometimes convenient to pour the entire mass through a sieve, rejecting the portion left in the sieve; or to wash the feces in a sieve, holding the latter under water. As a rule, however, the sieve is not very useful in fecal examinations.

The centrifuge does not appear to be of any special value in fecal examinations.

If facilities are not at hand for making a microscopic examination, about half an ounce of either perfectly fresh feces, or of rather dry feces, may be placed in a bottle, preferably with a large neck, properly packed in a mailing case, and sent to any professional pathologist or zoologist for examination.

GROSS EXAMINATION OF FECES.

If uncinariasis is suspected and it is not practicable either to make a microscopic examination or to delay matters until a specimen can be sent away for examination, still another method of diagnosis is possible. Give a small dose of thymol, followed by salts, and collect all of the stools passed. Wash the stools thoroughly several times in a bucket, and examine the sediment for worms about half an inch long, about as thick as a hairpin or hatpin, and with one end curved back to form a hook. If these are found, institute definite treatment.

TREATMENT OF HOOKWORM DISEASE.

ANTHELMINTHIC TREATMENT.

The two drugs most commonly used in uncinariasis are thymol and male fern. The day before treatment the patient is placed on a milk and soup diet for three days.

Thymol.—The directions usually given for thymol treatment are these: Two grams (31 grains) of thymol at 8 a. m.; 2 grams (31 grains) at 10 a. m.; castor oil or magnesia at 12 noon.

One week later the stools should be examined, and if eggs are still present, treatment should be repeated until the eggs disappear, but it is not best to give the thymol more than one day per week. Some cases of hookworm disease are quite obstinate and require a treatment extending over several weeks. It is, therefore, an unfortunate error to expel a few worms with one or two doses and then dismiss the patient as cured without having made further microscopic examination for eggs.

Sandwith (1894, p. 21) reports 42 men cured after a single dose; 58 after 2 doses; 43 after 3 doses; 25 after 4 doses; 9 after 5 doses; 4 after 6 doses; 2 after 7 doses, and 2 after 8 doses.

A number of writers, particularly Giles, Sandwith, and others, agree that small doses of thymol are valueless, but Sandwith is of the opinion that 4 grams in 24 hours are as efficacious as 6 grams, and the former dose is certainly less dangerous.

Worms may be found in the stools as early as eight hours after the first dose of thymol. In 50 cases Sandwith counted 1,301 worms in stools passed within eight hours after the first dose and 444 worms within the next sixteen hours. From his context, however, it is clear that brandy was given to these patients (see below), hence the thymol was dissolved more rapidly. Giles reports that he seldom found any worms until twelve hours after the first dose of thymol. "Occasionally patients vomited after swallowing thymol, but as a rule they retained it perfectly," and "they used to ask for an extra dose of it while convalescing."

Sandwith goes on to say that "large doses of thymol have a poisonous effect on the system, not unlike those produced by carbolic acid. The temperature is lowered one or even two degrees centigrade, and both pulse and respiration are slowed. The patient remains for a few hours collapsed, giddy, and faint, and has to be kept lying down, but at sunset he is quite well again and asking for food." He gives the following as a typical case:

. "January 14, 1892.—6 a. m., temperature 37.5°, pulse 80, respiration 19; patient in his usual state and was given 2 grams of thymol. 7 a. m., temperature 37°, pulse 80, respiration 19; says he has slight nausea, giddiness, and colicky pains in the epigastrium. 8 a. m., 2 grams more of thymol given. 9 a. m., temperature 35.5°, pulse 70, respiration 17; great giddiness, can not stand or walk; very sleepy, and talks like a drunken or very sleepy man. 12 a. m., symptoms much the same; sweating while asleep. 2 p. m., temperature 37.5°, pulse 75, respiration 18; apparently quite well again. Says he does not mind the thymol, except that it makes him lose consciousness."

Some authors advise the use of alcohol with thymol, others warn against such use. Sandwith, in referring to this subject, says: "Warned by the death of at least one of my patients immediately after digesting the thymol, I have always administered to feeble men 25 grams of brandy with each 2 grams of thymol, with the happiest results."

Authors who warn against the use of alcoholics during treatment do so on the ground that thymol is soluble part for part in alcohol, hence will more quickly be absorbed by the system. With one of my assistants (see Stiles & Pfender, 1902a), in the Bureau of Animal Industry, I treated a number of dogs with thymol in various forms. We gave doses varying from 10 to 100 grains (0.648 to 6.48 grams) to dogs weighing from 8 to 35 pounds. In alcoholic solution, 30 grains (1.94 grams) caused convulsions in a dog weighing 15 pounds, and severe convulsions in one weighing 14 pounds; on the other hand, an 8-pound, old dog suffered no ill effects after 30 grains in alcohol, and a 35-pound

dog took 75 grains (4.86 grams) in alcohol without deleterious effect. In tablet form, without oil, a single dose of 100 grains (6.48 grams) killed a dog of 15 pounds in four days; a dose of 50 grains caused a 12-pound dog to attempt to vomit; a dose of 75 grains was without appreciable effect in a dog of 35 pounds; 30 grains were without effect on a dog of 8 pounds; daily doses of 20 grains each caused a slight diarrhea after six days in a dog of 15 pounds; the same dose caused a 17-pound dog to be quite sick on the sixteenth day. In tablet form, with castor oil, 30 grains caused a 12-pound dog to attempt to vomit, while doses of 10 and 20 grains were negative on dogs weighing 12 to 15 pounds. In powdered form, without oil, 20 grains caused a 12-pound dog to attempt to vomit, while doses of 10 and 20 grains respectively were negative in dogs of 12 and 15 pounds; 40 grains caused no ill effects in a dog of 8 pounds, and a dose of 80 grains was without effect on a dog of 35 pounds. In powdered form, with castor oil, 20 grains caused slight convulsions in a dog weighing 15 pounds, but doses of 10 and 20 grains were negative on dogs of the same weight. Blue foxes to which we gave 3 grains in alcohol became quite sick. Theobald states that a dose of 3 grains has produced partial prostration in a bull-terrier, but he does not give the weight of the dog.

According to Sandwith, the contra-indications for thymol are "excessive debility, very low temperature, age above 60, and advanced diseases of the heart or any other organ. Boys take it very well in half quantities."

Sandwith states (1894, p. 17), that of 8 fatal cases treated with thymol 2 died, he thinks, in consequence of the thymol, eleven and forty-eight hours, respectively, after taking the dose; both of these men had previously had thymol without bad effect, but they were both in a miserable state of exhaustion and debility. He does not think that thymol accelerated the deaths of any of the remaining 6 cases, which occurred five, six, nine, thirteen, fifteen, and nineteen days, respectively, after the last dose of thymol. Three of these cases were over 65 years of age.

While my experience with thymol in man is very limited, I must confess that from my experiments upon animals, I am afraid of the use of alcoholics *per os* during treatment, and in the case of weak patients I should be more inclined to use a stimulant hypodermically than run the risk of dissolving the thymol too rapidly or in too great quantity at one time. Furthermore, it is at least doubtful whether our American hookworm will be so difficult to expel as is the Old World species, because of the absence of the ventral hooks (cf. figs. 5 and 10) in *Uncinaria americana*.

In the case of "excessive debility" and other conditions which Sandwith designates as contra-indications for thymol, it is not clear what drug Sandwith would use, for he states (1894, p. 20) that "for

the last three years I have looked upon it as a waste of time to administer any other anthelmintic than thymol for this parasite" [*Agchylostoma duodenale*]. Certainly persons in the conditions described should not be allowed to go untreated, and despite the view advanced by some authors, smaller doses of thymol, repeated one day per week and extending over several weeks, may be expected to yield some results.

Male fern.—Several European authors advise the use of large doses of extract of male fern in treating hookworm disease. Eichhorst's (1901, p. 314) recent "Practice" places the dose at 10 grams (2.5 fluid drams) to 20 grams (5 fluid drams). It has been pointed out by Lépine (1891a, 1891b) and others that such large doses of this drug are likely to be followed by serious toxic symptoms and even by death. Lépine summarizes the conditions as (1) symptoms of gastrointestinal irritation characterized by the redness and the hemorrhages; (2) nervous symptoms (convulsions and paralysis); (3) albuminuria, and (4) glycosuria, and he warns not to give over 8 grams of the extract as a maximum dose. Hare gives the dosage of the extract as 4 to 8 grams (about 1 to 2 fluid drams). Male fern should be followed in three to four hours by a calomel purge, aided by a saline, but not by castor or other oils, as the latter increase the danger of absorption, hence of poisoning.

Calomel.—While thymol is at present considered the most reliable remedy in hookworm disease, indications are not lacking (see p. 76) that considerable good may be accomplished in the American form of the disease by the use of calomel. This drug will not, however, be followed by such prompt and satisfactory results as will thymol.

GENERAL TREATMENT.

The administration of thymol has for its object the expulsion of the parasite, hence the removal of the cause of the disease. This should be supplemented by efforts to build up the depleted system by means of good nourishing food, iron, etc. It is well to give the iron daily, except on the days that thymol is taken. Sandwith (1894, p. 25) claims that the blood was most benefited by a daily supply of 1.5 grams (23 grains) of the sulphate of iron in water in three equal doses.

PROGNOSIS.

Among physicians I found the view rather prevalent that the prognosis was poor for children who presented severe cases of the disease. This view is probably due to the fact that the cause of the trouble was not understood, hence treatment was not directed to removing the cause.

The proposition now before us is, first, to remove the intestinal parasites, and second, to build up the patients.

To accomplish the first desideratum may require some patience, but efforts will eventually be successful. The second point may also be carried out, unless, of course, the patient is too far gone at the time of treatment to recover from the effects of the disease.

In not all cases can it be expected that a dwarfed, emaciated, and stupid child can be immediately placed upon the same physical and mental basis as his brothers, but even such cases can be greatly improved.

LETHALITY OF HOOKWORM DISEASE.

I know of no extensive and exact statistics regarding the lethality of uncinariasis, and traveling as rapidly as I did, it was impossible to establish any definite facts from personal observation, since it was the exception that I saw any case more than once. Furthermore, owing to the fact that many light cases will escape attention, any lethality percentages published will probably be above the actual figures.

I doubt whether sufficient data are at hand to justify even approximate statements regarding the lethality of hookworm disease. That numerous cases, not properly treated, terminate fatally can not be doubted. Still, it is remarkable how low a person may be with hookworm disease and still live.

Among physicians I met with the most contradictory ideas on this subject. Several excellent observers maintained that all severe cases which reached an edematous condition were invariably fatal; equally keen observers doubted whether this disease was frequently the actual cause of death; the view was quite general that patients suffering from medium or severe attacks of this malady very seldom lived through even medium or light attacks of such diseases as typhoid fever, or pneumonia, and that severe attacks of malaria were frequently fatal; also that they were very uncertain patients in confinement.

In my own observations, several points seemed quite significant. In the first place, the large number of cases of long standing found in so many families did not indicate a high lethality. Further, several adults were seen who had formerly unquestionably presented severe infections, but who are now in a fairly good state of health. In one family with 9 children living, most of whom clearly presented light, medium, or severe infections, there was a history of death of 9 other children, but satisfactory answers as to the cause of these deaths were not obtained. That some of the children had died of uncinariasis is very probable. On the other hand, families were seen with 8 to 10 children, all or nearly all in an anemic condition, some with clear medium to severe cases of uncinariasis, yet without history of any fatal case in the family. I have heard of localities in Central America (but have not investigated them personally) where it is said that a dis-

ease, which from its description I believe to be uncinariasis, probably due to *Uncinaria americana*, causes an immense mortality among the children.

Taken all in all, the data obtained did not convince me that uncinariasis, *per se*, is so fatal a disease in man as is generally supposed. On the other hand, I obtained the impression that while very severe cases are not infrequently fatal, the general effects of the malady upon the system are of greater and more far-reaching importance than the lethality of the infection itself. In other words, if uncinariasis were eliminated, the lethality of other diseases, such as pneumonia, typhoid fever, malaria, and also of child birth, would be decreased, and in the sand and mixed sand and clay areas this decrease would not be an insignificant factor.

One physician stated to me that he was confident that he had lost several hundred patients from uncinariasis within the past forty years.

Sandwith (1894, pp. 16-17) states that of the patients nominally under his care, 89.5 per cent were cured or greatly improved, 2.5 per cent were unrelieved, and 8 per cent died.

"Most of the fatal cases had loud anemic murmurs, marked subnormal temperature, slight general edema, albuminuria, and great mental weakness.

"The actual cause of death was exhaustion, from utter absence of rallying power. It is difficult to believe that the pathological effects are induced only by hemorrhage from the daily suction of scores or even hundreds of worms. In addition to the loss of blood, we have general thickening and degeneration of the duodenum and jejunum, and consequent interference with normal digestion; then nonassimilation, and eventually a process of slow starvation. It is also worthy of consideration that there may be in prolonged cases some self-poisoning from the great number of bites in the walls of the intestines containing ill-digested and perhaps decomposing food."

POST-MORTEM APPEARANCES.

I did not have occasion to make any autopsies during the trip; hence I am unable to present any original observations in this line. For careful accounts of single cases of autopsies, the reader is referred in American literature to Strong (1901), Yates (1901), Claytor (1902a), and Capps (1903a).

Sandwith's (1894, pp. 17-20) summary of 26 autopsies is not accessible to many American physicians; hence it is quoted here in full.

"Some of the earlier autopsies were made by myself, the later ones by Dr. Kaufmann. The muscles were in one case described as of normal color, but in all others they were very pale. There was usually a great absence of subcutaneous fat. The lungs were very pale and edematous in all cases, and all the organs were extremely bloodless.

"In one case there was noted edema of glottis. The heart was found to be hypertrophied ten times [in 10 cases], and was very small twice, generally pale brown in color, and on three occasions there were marked changes in the mitral valve. The most common abnormality in the liver was a brownish-yellow fatty appearance.

In one case there were several abscesses and the liver weighed 3,700 grams, and in one patient there was well-marked jaundice.

"The spleen was enlarged in one-third of the post-mortems. The kidneys invariably showed some change, though this was often much more marked in one kidney than in the other. They were very pale in 24 patients, 3 of whom had several small cysts. Of the remaining 2 patients one had granular kidneys and the other had cysts, but the kidneys were of normal color.

"The brain was always exceptionally white, and in one case there was recent apoplexy.

"The small intestines showed, of course, the most important changes. As a rule, there were many hemorrhages and bites in the jejunum and ileum, but in one of the cases where the bites were carefully counted there were only 6 in the jejunum and ileum, and no worms were found. In another case, however, there were 575 bites in the small intestine, besides 250 anchylostoma. In yet another there were 100 bites, the farthest of which was 4.5 meters from the pylorus. In only two cases was there much liquid blood in the intestine.

"I have not observed the constant changes in the mucous membrane of the stomach described by Giles. The great variability in the number of anchylostoma found at the autopsies is interesting. In 7 cases, all treated by thymol during life, no worms could be found. In an eighth case, also treated by thymol, but insufficiently, there were 10 worms. The remaining 18 cases had not had the advantage of thymol. Six of them, nevertheless, were found to have less than 10 worms, and in two of these corpses only 1 worm was found in each jejunum. Three other cases numbered 20, 40, and 50 worms, but the remaining 9 had numbers varying from 170 to 381, terminating with the maximum record of 863. On that occasion the autopsy was made seven hours after death, and the worms were scattered from a point 1 inch beyond the pylorus for the length of 3 meters; 217 of the 863 were attached still to the intestine and were surrounded by much bloody mucus, while 646 were lying free in the intestine; 16 of the latter were still alive, and one couple were in copulation.

"Next the position of the worms deserves notice. It was quite the exception to find any parasites in the duodenum. Can it be that when the duodenum becomes thickened and riddled, as it were, with the ravages of former generations, the anchylostomum fastens by preference on to the jejunum? Some such cause as this suggestion of gradually shifting the pasture is required to explain the interesting fact that it is not the most advanced cases of anemia which will always yield the largest quantity of worms. In such cases the parasite must not only have to burrow extra deep, but the blood when reached is, of course, deficient in quantity. Or is it that the half-starved worms are dislodged by repeated attacks of diarrhea? The furthest feeding ground that I have seen was 6.30 meters from the pylorus, where there was a worm firmly attached." But, as a rule, the attached worms are all within 2 meters of the pylorus, and have their heads and sometimes half their bodies buried in the mucous membrane. It is often impossible to dislodge them by a strong stream of water, and they must then be pulled out by forceps.

"I examined about 50 cases to see the proportion of male to female worms, and found it 56 to 44 per cent. This is not in accordance with some of the authorities, who say that males are always more rare than females, and that males are less influenced than females by the action of expellent drugs."

"Dubini has only once seen the worm in the ileum. The nearest feeding ground seen by me was 24 centimeters from the pylorus. In that case there were 100 worms attached and 281 detached, besides oxyurides."

PREVENTION OF HOOKWORM DISEASE.

In the prevention of diseases caused by animal parasites, we may, of course, attempt to attack the infectious agent in any stage of its life history. In connection with uncinariasis, three periods in particular come into consideration, namely: (1) The adult worm in the intestine; (2) the egg in the feces, and (3) the infecting ("encysted") stage of the larva.

(1) ADULT WORM IN THE INTESTINE; TREATMENT.

The destruction of the adult worm in the intestine not only relieves the patient of an important and (when present in large numbers) serious or even dangerous parasite, but it is also an important factor in preventing the spread of the disease to other people. Accordingly, treatment should be instituted even if the eggs found in the feces are so few in number as to indicate only a light infection.

Not infrequently the opinion is expressed that the infection with parasites found in a given patient is so light that treatment is hardly necessary. Such a view, however, is often very shortsighted, for it is not infrequently light infections occurring at unfavorable seasons and under unfavorable conditions that furnish the material for heavy infections at more favorable times. No *Uncinaria* infection in man is too light to be worthy of treatment, for each adult female may lay eggs; hence the destruction of these females means the decrease of scores of free infectious larvæ.

Not all cases of the malady can be recognized without the microscope; hence many people will unconsciously spread the disease-producing agent. Furthermore, many cases which might be recognized by symptoms will not come under medical treatment, so that they, too, will spread the infectious material. It is clear, therefore, that for satisfactory results in prevention we must adopt some method in addition to the treatment.

(2) EGGS IN THE FECES; CONTROL AND DESTRUCTION.

It is in the feces that we find the potentially infectious material in the most concentrated form. After the eggs develop into embryos the latter may leave the fecal matter and be distributed in the sand or in the water. Accordingly, it is much easier to control or destroy a given amount of infectious matter while it is concentrated in the feces than it is later when it is spread over a larger area. Here, in fact, we have the key to the prevention of uncinariasis. Proper disposal of the fecal discharges will make the spread of uncinariasis impossible. As such proper disposal I will suggest: Properly built privies when sewerage is lacking; use of such outhouses after construction; cleaning the same at regular intervals, and burial, burning, disinfection, or drying of the feces.

THE METHOD OF "DISINFECTING" PREMISES BY THE LAVA.

The method of "disinfecting" premises by the use of the lava is a chemical disinfection of premises to kill the eggs of the parasite, and also the parasite would



FIG. 80.—Spraying with burning oil, to kill superficial infection on premises where chemical disinfectants can not be used. (After Forbush & Fernald, 1900.)

be practicable, but heat, dryness, and cold all result in killing organisms.

About twenty-four to forty-eight hours of freezing temperature kills the free infection, hence after any cold weather of this kind in winter it may be assumed that the premises are disinfected.

After any especially dry weather, most if not all the free infection (except such as exists in places not affected by the dryness) is killed, so that exposed portions of premises may be assumed to be practically disinfected.

Spraying with burning oil (fig. 86), as practiced by the Massachusetts Gypsy Moth Commission, will effectually disinfect any area. If a spray nozzle or "cyclone burner" is not at hand, the ground around the house could be strewn with straw or brush and set afire (due precaution being taken not to burn the house), thus thoroughly disinfecting the premises. (See Stiles, 1902 d.)

Drinking water.—To tell the average farm hand or miner that he should always "boil or filter" the water before drinking it is, academically, a step toward preventing infection with uncinariasis. Practically, however, it is a step toward throwing away whatever influence we may happen to have with him. Theoretically, we should teach this simple hygienic precaution to all families, both in the city and in the country. Practically, we are in many cases weakening our position by insisting too generally upon this point.

While, therefore, we may warn people to boil or filter their drinking water in order to prevent the introduction of the infecting agent of uncinariasis or of other diseases, provided we see any chance of their following the advice (in regard to which we ourselves, except in times of epidemics, are very inconsistent), we will, I believe, usually weaken our influence with the poorer classes in mentioning a precaution which the average farm hand naturally looks on as absurd. It is much more important to urge him to locate his privy some distance from the well. That is a proposition he can appreciate; the necessity for boiling or filtering drinking water is usually beyond his mental horizon.

Clean hands.—An important point in connection with preventing the ingestion of the infectious agent of uncinariasis is that the hands and finger nails should be kept clean. I am inclined, however, to take an ultrapractical view of cleanliness versus dirt in connection with country houses, and to first see that the inevitable dirt shall be clean. This can be accomplished if we can succeed in having properly constructed latrines, built at proper distance from the wells and houses, if the children be taught to use them, and if the parents be taught the necessity for cleaning them.

These, in my opinion, are the first steps to be taken, and far outweigh all such considerations as boiling and filtering drinking water or keeping the hands clean.

Wearing shoes.—Wearing shoes during wet weather and washing the feet frequently will prevent the cutaneous infection and will protect to

a great extent against ground itch. It can hardly be expected, however, that the poorer children in country districts will adopt this precaution to any extent.

COMMON INTERPRETATION OF HOOKWORM DISEASE.

Upon several former occasions I have referred to "cases of anemia of obscure origin" as possibly due to uncinariasis. In a recent paper (Stiles, 1902b, pp. 207-208) I referred to "dirt-eating" as being possibly connected with uncinariasis; it was also intimated (1902b, p. 215), upon authority of Dr. Kirby-Smith, that in Mississippi uncinariasis is confused with malaria; Harris (1902c) also points out that much of the anemia attributed to malaria and dirt-eating is probably due to hookworm disease.

At present I am able to make more specific statements than formerly. The condition which should be attributed to light infections of uncinariasis is usually interpreted as due to malaria or diarrhea; medium cases are usually interpreted as an anemia due to malaria combined with "improper diet" or "insufficient nourishment;" severe cases are usually attributed to "malarial cachexia," "dirt-eating," "resin-chewing," "heart disease," "dropsy," "general debility," "pernicious anemia," and "lack of proper nourishment." Such at least are the most common diagnoses which have been made by the attending physicians in the cases which I have interpreted as light, medium, or severe infections with *Uncinaria americana*.

ECONOMIC IMPORTANCE OF HOOKWORM DISEASE.

Malaria is admittedly one of the most important diseases when viewed from an economic standpoint. In general, uncinariasis is, in the South, fully as important as malaria, and in some respects it is of even greater importance.

Take a given farming area in the sand district with an infection of uncinariasis, and assume that 100 farm hands are employed. It is not an exaggeration to say that these 100 people are not doing the work of 80 or 90 average hands. Thus there is a distinct loss of 10 to 20 per cent in the wages and a corresponding loss in the crop returns. In some places I should estimate the loss at even a higher percentage, say an average of 25 per cent, while in several families which I have examined I should say that uncinariasis is reducing the laboring capacity, hence the productiveness, of the family to as low as 30 to 40 per cent, thus entailing a loss of 60 to 70 per cent.

Nor are the losses in wages and in the laboring capacity, and the decrease of productiveness of the family, hence of the farm, and finally of the county and State, the only economic considerations involved. Cases are not unknown where families have sold, moved, or destroyed their homes, or were about to do so, because of the existence of this disease and because of the belief that it might be due to the locality in which they lived.

Again, it is almost a common experience to be told by the father of a family that he spends for medicine all he earns, in the hope of ridding his children of this malady. Add to this the physicians' bills, the loss by death and funeral expenses, etc., and it is seen that this infection is keeping more than one family in absolute poverty.

Nor should we forget that uncinariasis has its important bearing upon the mental as well as upon the physical and financial development of the poorer white people. As already stated, children infected with this malady are often underdeveloped mentally; frequently they have a reputation in the schools, in the neighborhood, and in their own family, of being "stupid," or "dull," or "backward" in their studies, etc. It has already been mentioned that children suffering with this disease are frequently kept home from school because of their tendency to become edematous when they sit still for any length of time. When we now recall that these conditions coincide especially with the educational period, it should not seem strange that uncinariasis has a marked influence upon the general intellectual condition of the districts in which it occurs.

Considering the subject in the light of all I saw on the trip, and taking what I believe to be a conservative view of the subject, I find it exceedingly difficult to escape the conclusion that in uncinariasis, caused by *Uncinaria americana*, we have a pathologic basis as one of the most important factors in the inferior mental, physical, and financial condition of the poorer classes of the white population of the rural sand and piney wood districts which I visited. This sounds like an extreme statement, but it is based upon extreme facts.

By this position I do not intend to assert that uncinariasis is the only factor which comes into consideration. The warm climate and the monotonous diet, and probably also the excessive use of tobacco in some cases, are not without influence. Still, with uncinariasis as it exists to-day, these people are suffering from a handicap in life which practically removes them from a fair chance in competition. If the uncinariasis is removed they will be placed in a more favorable condition both subjectively and objectively. With the present prevalence of uncinariasis their lack of ambition is perfectly natural; remove the disease and they can develop ambition.

On the other hand, if we were to select the strongest people in the country and place them in the conditions under which these patients are now living it would be only a generation or two before even a race of athletes would be in the same condition as the persons under discussion.

The conditions described are familiar to persons who have visited the rural sand districts. But they have existed for so many years that many of us to-day look upon them as natural, hence they do not attract the consideration to which they are entitled.

GEOGRAPHIC DISTRIBUTION AND ABSTRACTS OF CASES FOUND IN THE UNITED STATES.

In a former paper (Stiles, 1902b, pp. 206-217) I gave abstracts of all the cases of uncinariasis known to me at that time for the United States. The disease is now proved to be so common in certain portions of the country that it is hardly necessary to keep a full record of every case found, but on account of the medico-historical interest associated with the subject, and also in order to complete the literature and details of geographic distribution, there are here added abstracts and notices of various cases which have come to my knowledge since the above-mentioned paper was completed.

NEW ENGLAND STATES.

NEW HAMPSHIRE.

No positively diagnosed cases of hookworm disease seem to be recorded for this State.

Center Eppingham, 1876 ? 1 case, ? death.

GOULD (1876, pp. 417, 418) refers to a case of pica or dirt-eating which sounds suspiciously like uncinariasis.

MIDDLE STATES.

NEW YORK.

Rochester, 1868 ? 1 case, ? death.

ELY (1868, pp. 101, 102) describes a case of chalk-eating which may possibly have been due to uncinariasis, though this is by no means certain.

Buffalo, 1896 5 cases, 0 death.

MÖHLAU'S (1897) cases. See STILES (1902b, p. 209). Doubts have arisen in the minds of some physicians as to whether these were actually cases of uncinariasis.

Glen Island, 1900 4 cases, 0 death.

ASHFORD'S cases from Porto Rico. Probably due to *Uncinaria americana*; reported in STILES (1902b, p. 210).

Stapleton 1 case, 0 death.

BAILHACHE'S, and GREENE'S (1901) case. Place of infection uncertain. See STILES (1902b, p. 215).

Albany, 1900 1 case, 0 death.

WARD (1902, pp. 23-26): American, physician, 32 years old. Had served in U. S. Army in the Philippines. Albany hospital, Feb. 6, 1902. Report on feces by Dr. GEORGE BLUMER, confirmed by Dr. W. S. THAYER.

? **Albany, 1900** 1 case, 0 death.

NEUMAN and BLUMER. Details of case not known to me.

PENNSYLVANIA.

It is by no means impossible that uncinariasis will be found among the miners of Pennsylvania. As so many of these men are immigrants from Europe, the Old World species, *Agchylostoma duodenale* may be expected.

Philadelphia, 1900-1901 3 cases, 0 death.

BOSTON'S cases reported by ALLYN and BEHREND (1901). See STILES (1902b, p. 211). Probably due to *Uncinaria americana*.

Philadelphia, 1901 1 case, 0 death.

ALLYN and BEHREND'S (1902) case, imported from Italy, hence due to *Agchylostoma duodenale*.

MARYLAND.

Baltimore, 1900 2 cases, 0 death.

HEMMETER'S (1902) cases; probably infected in Porto Rico and due to *Uncinaria americana*. See STILES (1902b, p. 210).

Baltimore, 1901 1 case, 1 death.

HAL'S (1901) case; imported, possibly from Vera Cruz. Due to *Agchylostoma duodenale*. See STILES (1902b, pp. 213-215).

Baltimore, 1902 1 case, 0 death.

OSLER'S case [unpublished]. In Johns Hopkins Hospital. Patient came from North Carolina. Parasites determined by Boggs as *Uncinaria americana*, confirmed by Stiles.

DISTRICT OF COLUMBIA.

Washington, 1901 [1 case, 1 death.]

CLAYTOR'S (1901a, 1902a) case, from Westmoreland County, Va., due to *Uncinaria americana*. See STILES (1902b, pp. 211-212).

Washington, 1902 1 case, 0 death.

HERRICK (1902, p. 101): Male, 37 years old, lived in Germany until 1897. Sent to Philippine Islands September, 1899. "Present illness began in July, 1900, with an attack of diarrhea. He had from 10 to 15 movements daily for three months, with a moderate amount of tenesmus. Mucus and blood appeared in the stools after the first month, giving them a dark tarry appearance. He lost weight and strength rapidly during this time and gradually became short of breath on slightest exertion. This was followed by a period of improvement, the stools becoming less frequent; but in January, 1901, he became worse and was sent to the hospital at Iloilo. Since then he has been confined to hospitals, on account of weakness and dyspnea. In September, 1901, the diarrhea ceased, and although he has gained a little weight he has been steadily growing weaker. At no time had he been subject to hemorrhages other than stated.

"Physical examination shows an apparently well-nourished man with a peculiar lemon-yellow pallor; conjunctivas and mucous membranes are pale; slight oedema of the ankles is present. He has marked dyspnoea on the slightest exertion. The lungs are negative; the heart is enlarged; the point of maximum impulse is in the fifth intercostal space in the nipple line. A soft blowing systolic murmur is audible at the apex and in the pulmonic area. Liver dullness extends from the sixth intercostal space in the nipple line to 1 cm. below the costal margin. The edge is palpable. The spleen is enlarged and the edge is palpable at the costal margin. The urine is negative.

"The blood is very pale and watery; a moderate poikilocytosis is present; there are no nucleated red corpuscles and no malaria organisms present. The blood count shows: Red corpuscles, 1,120,000; hemoglobin, 18 per cent; leucocytes, about 4,000; polymorphonuclear, 52 per cent; eosinophiles, 26.8 per cent; small mononuclear, 14 per cent; large mononuclear, 4.4 per cent; transitional, 2.8 per cent.

"One month later, the patient meantime having been taking arsenic and iron, the blood count was as follows: Red corpuscles, 1,450,000; hemoglobin, 22 per cent; leucocytes, 2,000; polymorphonuclear, 61 per cent; eosinophile, 18.2 per cent; small mononuclear, 16.4 per cent; large mononuclear, 3.2 per cent; transitional, 1.2 per cent; no nucleated red cells.

"There had been practically no change in the patient's condition. The liver and spleen were as in the former note, and the dyspnea was marked. Numerous typical ova of the *Uncinaria* were present in the stools, but no adult forms were seen. After the usual thymol treatment about 60 adult worms were found. They resembled in all respects *Uncinaria duodenalis*, and were identified by Dr. Stiles as the Old World hookworm. The ova present were in the progress of segmentation, 4 to 12 cells being visible. None were seen containing an embryo, as frequently occurs in the form *Uncinaria americana*, described by Dr. Stiles.

"Blood examinations, ten and twenty days after the thymol treatment was begun, showed the following counts:

	May 28, 1902.	June 7, 1902.
Red corpuscles.....number..	2,300,000	3,100,000
Hemoglobin.....per cent..	23	27
Leucocytes.....number..	2,500	3,000
Polymorphonuclear.....per cent.	54	62
Eosinophile.....do.....	21	14
Small mononuclear.....do.....	17	17
Large mononuclear.....do.....	6	6
Transitional.....do.....	2	1

"The general condition is also improving rapidly, although the parasites are not entirely eliminated, as an ovum is still occasionally found in the stools."

Anacostia (Government Hospital for the Insane), 1902. 16 cases, 0 death.

Cases found on microscopic examination by Stiles, Garrison, Ransom, and Stevenson, of United States Public Health and Marine-Hospital Service. Probably most if not all of these were infected in other localities. (See p. 37.)

VIRGINIA.

Essex County, ? date ? cases, ? deaths.

Passed Asst. Surgeon JOHN F. ANDERSON has stated to me that there exists in Essex County a condition of "bloat" and anemia which is usually attributed to dirt-eating, and which corresponds in general to the conditions described in this paper.

Richmond, 1852 ? 1 case, 0 death.

POLLARD (1852, p. 185) reports a case of dirt-eating. Its connection with uncinariasis is possible, but not clear.

Richmond, 1898 1 or 2 cases, 0 death.

GRAY'S (1901) case. See STILES (1902b, p. 209).

Westmoreland County, 1901 1 case, 1 death.

CLAYTOR'S case. See District of Columbia.

Westmoreland County, ? date 2 cases, 0 death.

Referred to by STUART in STILES (1901, p. 525, and 1902b, p. 212).

NORTH CAROLINA.

Judging from the size of the eggs, all the cases I found in North Carolina were due to *Uncinaria americana*.

Roanoke River Valley, prior to 1808.....? cases, ? deaths.

PITT (1808) states that malacia or dirt-eating "prevails mostly among the poorer white people and negroes, and originates in my opinion from a deficiency of nourishment." He refers also to the slowly healing ulcers on the legs, and to the "tallow complexion." His general description points quite distinctly to uncinariasis.

Person County, 1832.....? cases, ? deaths.

JORDAN (1832, pp. 18-30) gives a discussion of dirt-eating which quite positively refers, at least in part, to uncinariasis.

Durgy, Person County, 1902.....2 cases, 0 death.

STILES (1903b, p. 38).

Cumnock Coal Mines, Chatham County, 1902.....1 case, 0 death.

STILES (1903b, p. 38).

Gaston County, about 1880.....? cases, ? deaths.

The following interesting letter has been received from Dr. Barringer, and indicates the presence of uncinariasis in Gaston County:

"DEAR SIR: I have just seen in the Marine-Hospital Service Public Health Reports your letter of October 22, from Kershaw, S. C. I was for many years located in the district in which you have been working, and your letter has thrown an immense amount of retrospective light on what I saw there. My work was done in the early eighties, and yet I still remember many cases of pernicious anemia, which was accompanied in some cases by dropsical effusions and diarrhea, a combination I could never make out, and yet this must have been uncinariasis.

"Whole sections of the illicit distillers of Kings Mountain, in Gaston County, N. C., were affected, and the dirt-eating whites of this section seemed to have a malady different from those of the better class in the neighborhood. I tried a tannic acid preparation, which seemed to do more good than anything else, and I wish now I had tried Areca nut. By the bye, I also recall that my pointer dogs in this section seemed to be afflicted in the same way. During my stay in Gaston County, from 1878 to 1881, I lost two dogs, who used to follow me around to these houses, from an unknown disorder.

* * * * *

"I remain, yours, very respectfully,

"P. B. BARRINGER, *Chairman*.

"DR. CH. WARDELL STILES,

"*Care Marine-Hospital Service, Washington, D. C.*"

SOUTH CAROLINA.

Judging from the measurements of the eggs, all the cases I found in South Carolina were due to *Uncinaria americana*.

Date?.....? cases, ? deaths.

HEUSINGER and GEDDINGS, quoted by BLANCHARD, 1888a, could not be traced.

Adams Run, Colleton County, 1902.....4 cases, 0 death.

STILES (1903b, p. 41): Orphans at Charleston.

- Barnwell County, 1902** 1 case, 0 death.
 STILES (1903b, p. 41): Medical student at Charleston.
- Berkeley County, 1902** 3 cases, 0 death.
 STILES (1903b, p. 41): Orphans at Charleston.
- Camden, Kershaw County, 1902** 2 cases, 0 death.
 STILES (1903b, p. 39): Brickyard.
- Charleston, Charleston County, 1902** 3 cases, 0 death.
 Dr. DE SAUSSURE, quoted by STILES (1903b, p. 41): Source of infection not stated.
- Charleston County, 1902** 2 cases, 0 death.
 STILES (1903b, p. 41): Medical students at Charleston; came from seacoast islands.
- Charleston, Charleston County, 1902** [15 cases, 0 death.]
 STILES (1903b, p. 41): At orphan asylum. Children came from Dorchester (1), Berkeley (3), Colleton (4), and Charleston (7) counties
- Charleston, Charleston County, 1902** [4 cases, 0 death.]
 STILES (1903b, p. 41): Medical students from Barnwell (1), Florence (1), and Charleston (2) counties.
- Florence, Florence County, 1902** 1 case, 0 death.
 STILES (1903b, p. 41): Medical student at Charleston.
- Lancaster and Kershaw counties, 1902** about 50 cases, 0 death.
 STILES (1903b, pp. 40-41).
- McClellanville, Charleston County, 1902** 4 cases, 0 death.
 STILES (1903b, p. 41): Orphans at Charleston.
- Plum Island, Charleston County, 1902** 3 cases, 0 death.
 STILES (1903b, p. 41): Orphans at Charleston.
- Summerville, Dorchester County, 1902** 1 case, 0 death.
 STILES (1903b, p. 41): Orphan at Charleston.

GEORGIA.

Judging from the size of the eggs, all the cases I found in Georgia were due to *Uncinaria americana*. Harris states that his cases were due to the same species.

Locality? Date? ? cases, ? deaths.

LYELL quoted by BLANCHARD (1888a), could not be traced.

Richmond County, 1836 ? cases, ? deaths.

COTTING (1836a, pp. 288-290) states that clay is eaten by many people, especially by children. Probably at least some of the cases were connected with uncinariasis.

Pine Barrens of Georgia, 1845 ? cases, ? deaths.

LE CONTE (1845, pp. 417-444) states that dirt-eating is common in the pine barrens of Georgia. His description refers quite clearly to uncinariasis, at least in part.

Appling County, 1902 1 case, ? death.

HARRIS (1902a, pp. 99-100): Male, farmer, 29 years old. Healthy until 14 years of age, then observed that he was never so well in latter part of winter and spring as in summer and fall. Anemic; weak; food tastes salty; in spring the arms, hands, and dorsal surface of feet become greatly inflamed, blisters form, followed by scabs; severe constipation; pains in neck and stomach; vomiting frequent; weight 117 pounds; skin pale and wrinkled, smooth and dry; very little beard; mucous membranes very pale; tongue moist, shows indentations of teeth, and its epithelium in a large measure absent; teeth small, quite a number of them decayed; pulse 90, respiration 20, temperature 98° F.; body somewhat emaciated; heart with soft, blowing systolic murmur constant, varying greatly in intensity; marked venous hum over right jugular; just below the ensiform cartilage and to the left great tenderness; stomach normal in size and position; after Ewald trial meal, total acidity 64, HCl 40, combined HCl 4, phosphates 4; spleen and intestines normal; feces dark brick-red; *Uncinaria* eggs present; urine 2,300 cm. in twenty-four hours, light yellowish-red; specific gravity, 1.012, faintly alkaline, no sugar, at one time faint ring of albumin, albumose not present; urea in twenty-four hours, 21.15 grams, uric acid 0.475 gram, chlorids 3.15, phosphates 2.37, sulphates 2.82 grams. Blood: Red corpuscles 1,760,000, white 4,020, hemoglobin 20 per cent; decided though not extreme poikilocytosis, a number of microcytes; a few nucleated reds; small lymphocytes 28, large lymphocytes 14, transitional 6, polymorphonuclear leucocytes 50, eosinophiles 2. Vision, right eye 15-20, left eye 15-30. Diagnosis: Anchylostomiasis and possibly pellagra. Treatment: Afternoon, 10 grains of calomel; next day, 7 a. m., 30 grains of thymol in capsules; 9 a. m., 30 grains of thymol; 8 p. m., large dose of salts. Stools contained at least 420 worms.—HARRIS, 1902b, pp. 220-227. Same case.

Porter Springs 4 cases, 0 death.

Letter of H. F. HARRIS, dated August 9, 1902, to U. S. Bureau of Animal Industry. He states: "I am absolutely sure that this disease is very common in all this region." One of the four cases probably originated in Troup County, the other three in Lumpkin or neighboring county.

Locality ?, 1902 7 cases, 0 death.

HARRIS (1902c, p. 776) states that since reporting his first case he has discovered eleven new cases for Georgia. See also Porter Springs.

Atlanta, 1902 1 case, 1 death.

CLAUDE A. SMITH (1902, p. 1062): Case reported; mentioned also a similar case in a dog which had eaten some of the infected feces of the patient. Man died of pleuritic abscess. The specimens were collected post-mortem in a negro at Grady Hospital, Atlanta.

Dr. Smith kindly sent me the parasites for examination. The specimens from man (B. A. I., No. 3423) proved to be *Uncinaria americana*, while those from the dog (B. A. I., Nos. 3424 and 3425) were *Agchylostoma caninum*.

Atlanta, November, 1902 2 cases, 0 death.

CLAUDE A. SMITH informed me in a personal letter dated December 3, 1902, that he had just observed two cases, both from Florida. One was a man 50 years old, the other a dental student.

Albany, Dougherty County, 1902 about 5 cases, 0 death.

STILES (1903b, p. 43): People did not belong in Albany.

Americus, Sumter County, 1902 1 case, 0 death.

STILES (1903b, p. 41): Orphan in Macon.

- Baxley, Appling County, 1902** 1 case, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.
- Buena Vista, Marion County, 1902** 2 cases, 0 death.
 STILES (1903b, p. 41): Orphan in Macon.
- Cordele, Dooly County, 1902** 1 case, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.
- Darien, McIntosh County, 1902** 1 case, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.
- Effingham County, 1902** 1 case, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.
- Fort Valley, Houston County, 1902** 50 cases, 0 death.
 STILES (1903b, p. 42): Dr. BROWN stated he could easily find 50 or more cases. We examined about 10 cases together.
- Jackson County, 1902** ? cases, ? deaths.
 Dr. HARDMAN, quoted by STILES (1903b, p. 41).
- Johnson County, 1902** 1 case, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.
- Jones County, 1902** 4 cases, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.
- Kinderlout Station, Lowndes County** 1 case, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.
- Lee County, 1902** 4 cases, 0 death.
 STILES (1903b, p. 43): Observed with Dr. HILSMAN.
- Lyon, Tattnall County, 1902** 1 case, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.
- Macon, Bibb County, 1902** [29 cases, 0 death.]
 STILES (1903b, pp. 41-42): At orphan asylums. See Americus (1), Baxley (1), Buena Vista (1), Cordele (1), Darien (1), Effingham (1), Johnson (1), Jones (4), Kinderlout (1), Lyon (1), Monroe (1), Richwood (2), Sandersville (1), Thomasville (1), Savannah (1), Waycross (3), Monroe (1), Georgia. Also: Deland (4), Liveoak (1), Wacissa (1), Florida.
- Macon, Bibb County, 1902** about 25 to 30 cases, 0 death.
 STILES (1903b, p. 42): About 25 to 30 cases; men, women, and children in the cotton-mill families.
- Monroe County, 1902** 1 case, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.
- Richwood, Dooly County, 1902** 2 cases, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.
- Sandersville, Washington County, 1902** 1 case, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.

Savannah, Chatham County, 1902 1 case, 0 death.

STILES (1903b, p. 42): Orphan in Macon.

Thomasville, Thomas County, 1902 1 case, 0 death.

STILES (1903b, p. 41): Orphan in Macon.

Washington County, 1902 ? cases, 0 death.

In personal conversation with Dr. A. MOODY BURT, I was informed that there were a number of persons in Washington County who show in general the symptoms of *uncinariasis*.

Waycross, Ware County, 1902 3 cases, 0 death.

STILES (1903b, p. 42): Orphan in Macon.

Waycross, Ware County, 1902 ? cases, ? deaths.

STILES (1903b, p. 43): Many cases, number not estimated; about 20 cases of *uncinariasis* to 1 of malaria; on authority of Drs. IZLAR and WALKER.

Willacoochee and vicinity, Coffee County, 1902 ... 200 cases, 0 death.

STILES (1903b, p. 43): Given on authority of Dr. WILCOX. We examined 8 cases together.

FLORIDA.

Judging from the size of the eggs all the cases I found in Florida, so far as examined microscopically, were due to *Uncinaria americana*.

Prior to 1845 ? cases, ? deaths.

LITTLE (1845) refers to dirt-eating; see STILES (1902b, p. 208).

Locality ?, 1902 ? cases, ? deaths.

GUITERAS's cases; see STILES (1902b, p. 215).

Locality ?, 1902 1 case, 0 death.

HARRIS (1902c, p. 776): Locality not given; originated in Florida.

Baker County ? cases, ? deaths.

In personal conversation with a Jacksonville druggist, I was informed that so-called dirt-eaters are common in Baker County.

Clay County ? cases, ? deaths.

In personal conversation with a Jacksonville druggist, I was informed that so-called dirt-eaters are common in Clay County.

Deland, Volusia County, 1902 4 cases, 0 death.

STILES (1903b, p. 42): Orphans in Macon, Ga.

Jacksonville, Duval County, 1902 2 cases, 0 death.

STILES (1903b, p. 44).

Liveoak, Suwanee County, 1902 1 case, 0 death.

STILES (1903b, p. 42): Orphan in Macon, Ga.

Tampa, Hillsboro County, 1903 12 cases, 0 death.

In a letter dated March 21, 1903, Dr. J. S. HELMS says: "I have to date collected 12 cases and am yet working. I dare say that there are hundreds of cases in south Florida." The parasites were *Uncinaria americana*.

Wacissa, Jefferson County, 1902..... 1 case, 0 death.

STILES (1903b, p. 42): Orphan in Macon, Ga.

Ocala, Marion County, 1902 5 cases, 0 death.

STILES (1903b, p. 44).

Twiggs County, 1902..... ? cases, ? deaths.

I have been informed that in Twiggs County there exists a condition which corresponds to uncinariasis.

Upson County..... ? cases, ? deaths.

There is said to exist a great deal of "bloat" in this county. Possibly this "bloat" is due, in part at least, to uncinariasis.

Waldo, Alachua County, 1902..... about 12 cases, 0 death.

STILES (1903b, p. 44).

ALABAMA.

? Locality..... ? cases, ? deaths.

LYELL, quoted by BLANCHARD, 1888a. Could not be traced.

Middle Alabama, 1902..... 1 case, 0 death.

HARRIS (1902c, p. 776).

Mobile and vicinity..... 24 cases, 0 death.

The following extracts are made from a letter dated March 3, 1903, from Dr. E. D. BONDURANT, professor of pathology, medical department, University of Alabama: "Some weeks ago a fellow practitioner told of cases of intense and protracted anemia he was treating, suggested the possibility of uncinariasis, and asked me to make a microscopic examination of the fecal discharges. This was done, and I had no difficulty in promptly identifying the hookworm ova in the feces of every one of his 4 cases. Shortly afterward I came upon 2 cases in my own practice, found the ova in quantity, and after thymol I found numbers of adult worms. At the city hospital we have already had several [?] others, and one physician who has actively taken up the search in his anemic country patients tells me that he has found about 15 cases. There is no doubt that the disease is *very common* in the country surrounding Mobile. * * * Our 'poor whites' are surely widely infected with the disorder * * *."

In a letter dated March 13, Dr. E. D. Bondurant says: "I have, since I last wrote you, diagnosed cases from Monroe County, Covington County, and Crenshaw County, this State, as well as numerous other cases in the district immediately around Mobile. * * * All of my cases have promptly improved after thymol."

Monroe County, 1903..... 1 case, 0 death.

Quoted on the authority of a letter dated March 3, 1903, from Dr. E. D. BONDURANT.

Waldo, Talladega County..... ? cases, ? deaths.

A trained nurse, Miss Edith Lide, has described to me a family at Waldo whose symptoms (anemia, heart, emaciation, dirt-eating, etc.) point almost unmistakably to hookworm disease.

MISSISSIPPI.

One of the American physicians (Dr. Kirby-Smith) who saw several cases of uncinariasis in Cuba has recently stated to me that this disease is undoubtedly present in Mississippi, but its exact nature has not been recognized. He is convinced that he himself has seen a number of cases which were confused with malaria.

ARKANSAS.

Uncinariasis does not appear to be proved as yet for Arkansas, but I have been told that it is probably present.

LOUISIANA.

Louisiana, prior to 1821 and 1850.....? cases, ? deaths.

CHABERT's (1821a) and DUNCAN's (1850, St. Mary Parish) accounts of dirt-eating apply very well to uncinariasis. See STILES (1902b, p. 207).

New Orleans, 1899.....1 case, 0 death.

TEBAULT's (1899) case. See STILES (1902b, p. 209).

Dirt-eaters are said to be numerous in the Mississippi Delta near Baton Rouge.

TEXAS.

Locality?, 1864.....1 case, 1 death.

HERFF's (1864) case in Mexican woman. See STILES (1902b, p. 208).

Galveston, 1894.....1 case, 0 death.

ALLEN J. SMITH's case, reported by SCHAEFER (1901). See STILES (1902b, p. 208).

Galveston, 1909-1901.....1 case, 0 death.

SCHAEFER's (1901) case; probably infected in Mexico. See STILES (1902b, p. 211).

Galveston, 1901.....8 cases, 0 death.

ALLEN J. SMITH's cases, reported in part by SCHAEFER (1901). See STILES (1902b, p. 211). At least one of these cases was due to *Uncinaria americana*.

Encinal, La Salle County; Heampstead, Waller County; Lavaca County, 1898.....? cases, ? deaths.

Upon seeing my description of hookworm disease and the photograph from which figure 42 was made, MR. CHARLES A. PFENDER, assistant in the Zoological Laboratory, U. S. Bureau of Animal Industry, stated to me that he had seen similar conditions among Mexican children in Encinal, among negroes at Heampstead in Brazos River bottom, and among the poorer people in the southern portion of Lavaca County, along the Navidad River.

CENTRAL STATES.

ILLINOIS.

Chicago, 1902.....1 case, 1 death.

CAPPS (1902a; 1903a, pp. 28-33): Patient, G. L., in Cook County Hospital; male; 52 years old; carpenter; American. Infection probably took place at Panama. Earliest symptoms two years ago, aching pain in upper belly, loss of appetite, and weakness. No nausea or vomiting. Bowels irregular, loose or constipated. Later, shortness of breath and dizziness; also palpitation of heart. In hospital fifteen months at New Orleans; diagnosis, pernicious anemia. September 9, 1902, admitted to Cook County Hospital; pains in belly, weakness, dyspnea, and palpitation. After sitting or standing feet would swell. Frequent dizziness and faintness. Occasionally had developed moderate fever, at which time epigastric pain was worse. Appetite poor. Constipation. Gradual, moderate emaciation.

"*Status presens*.—Man of medium build; skin of a lemon-yellow color. Fatty layer fairly well preserved. Sclerotics bluish and muddy. Lips and mucous membranes almost bloodless. Palpable arteries moderately thickened. Pulse of large volume, soft and compressible; low tension, with a decided water-hammer character. Lungs negative. Heart dullness extended to the left nipple line, to the upper border of the third rib above, and to the right edge of the sternum. The impulse was forcible and diffuse. Over the apex was a systolic blow transmitted to the anterior axil-

lary line. A louder bruit of a different pitch was audible over the mitral area and the base. The pulmonic sound was louder than the aortic closure.

"The spleen was not palpable, though its area of dullness was enlarged. The liver was not felt. The epigastric and umbilical regions were tender to pressure. Knee jerks present but not prompt.

"Temperature 98.6, pulse 90, respiration 20. Urine 1.015; no albumen, no sugar, and no casts.

"September 11, blood examination showed hemaglobin 18 per cent; reds, 2,576,000; whites, 6,600. No stained preparations were made.

"September 15, gastric contents were expressed one hour after a test meal of tea and toast. No free HCl present; no lactic acid.

"September 23, blood examination, hemog. 17 per cent; reds, 2,280,000; whites, 6,000.

"October 20, blood examination, hemog. 12.5 per cent; reds, 843,000; hematocrit, 980,000; whites, 4,500; color index, 0.80; volume index, 1.17. The differential count showed: small mononuclear, 10 per cent; large mononuclear, 11 per cent; polymorph. neutrophile, 66 per cent; polymorph. eosinophiles, 13 per cent; no rouleaux formation; no nucleated red cells; poikilocytosis marked; polychromatophilia marked.

"The presence of pronounced eosinophilia in a case of grave anemia made us strongly suspect the existence of an intestinal parasite, so that the stools were examined frequently. The earlier specimens of feces were watery from the rectal injections employed, and were therefore not easily studied. In a formed movement, however, the ova were found in large numbers. These eggs corresponded accurately in dimensions to those of *Uncinaria duodenalis*, measuring about 56 microns in length and 34 microns in width.

"Subsequently the eggs of *Tricocephalus dispar* were found in small number by Dr. J. L. Miller. Charcot-Leyden crystals were present in some preparations, absent in others. Cover glass smears of feces hardened in alcohol and ether were stained with hematoxylin and eosin and eosinophilic granulations demonstrated. These granulations, like the Charcot crystals, were never numerous, as is so often the case in ankylostomiasis.

"The eggs were successfully cultivated and the larvæ brought to mature development. These experiments will be described later.

"November 6, examination of the blood gave: hemaglobin 11 per cent; reds, 748,000; hematocrit, 915,000; whites, 5,600; color index, 88 per cent; volume index, 122 per cent. Differential count: small mononuclear, 15.8 per cent; large mononuclear, 6.8 per cent; polymorph. neutrophile, 70.2 per cent; eosinophile, 7.6 per cent; eosinophilic myelocytes, 0.2 per cent; mast cells, 0.4 per cent. Poikilocytosis and polychromatophilia marked. Coagulation time, five minutes.

"Previous to this examination the patient had taken thymol in small doses, and it is not unlikely that many parasites were swept away and lost in the stools. The diminished eosinophilia and the small number of eggs found in the stools thereafter lend support to this assumption.

"History in the hospital.—Treatment, on the whole, was unsatisfactory, because of the profound weakness of the patient and the irritable condition of the stomach. A persistent nausea set in that interfered with stomach feeding and made the administration of thymol ineffective and even hazardous. Nutrient and salt enemata were resorted to, but the vomiting persisted until the patient succumbed, on November 13.

"During his stay in the hospital the most conspicuous symptom was epigastric pain of a dull nature at first, subsequently colicky. In the last two weeks this pain became continuous, and was accompanied by a great tenderness over the epigastric and right hypochondriac regions. The liver mass extended at this time about 2 inches below the costal arch, and the tenderness, on pressure, was as great as that seen in hepatic abscess.

"The bowels, as a rule, were constipated, and required rectal enemata. The tem-

perature was usually normal, and ranged between 98.5° and 99.5° F. A tendency to hemorrhage was nowhere to be seen, except from the intestinal tract. The feces gave the prussian-blue reaction for iron.

"The examination of the eye grounds was twice carried out under difficulties. No retinal hemorrhage was apparent.

"The blood findings throughout were of a most suggestive nature; an anemia, at first of the secondary type, progressing until it corresponded in most respects to a primary pernicious anemia. The individual corpuscles grew larger and held an ever-increasing amount of hemoglobin, the color index rising from 38 to 88 per cent, and the volume index reaching 122 per cent. The poikilocytosis was sufficiently outspoken, as well as the polychromatophilia, for a primary anemia. The scarcity of nucleated red corpuscles and the entire absence of megaloblasts is unusual in the primary pernicious form, yet some such cases have been reported. What convinced us that the anemia was not of the usual primary type was the eosinophilia of 13 per cent, for in the primary disease the eosinophiles are rarely increased. On the other hand, the Uncinariæ and most of the other intestinal parasites are characterized by an increase in the eosinophilic cells.

"*Autopsy.*—The post-mortem examination was made November 13 by Dr. Harris, resident pathologist of the hospital. His report is as follows:

"Body is that of a fairly well-developed and fairly well-nourished man 162 cm. tall. Post-mortem rigidity and lividity present.

"There is a scar 0.5 cm. long on left arm, some edema of lower extremities. Panculus adiposus well preserved and of a light-yellow color.

"Abdominal cavity: Diaphragm reaches to the fifth rib on the right side and to the sixth rib on the left. Some free straw-colored fluid in peritoneal cavity. Omentum extends down to the pelvis over the intestines and contains a considerable quantity of fat.

"Pleural cavities: Some firm fibrous adhesions at right apex. About 1,000 c. c. straw-colored fluid in right pleural cavity; about 250 c. c. in left. Lungs do not meet in median line.

"Pericardial cavity: About 500 c. c. of straw-colored fluid in the pericardial cavity. This fluid contains a few fibrinous flocculi. The pericardium is thin and surface is smooth.

"Tongue, pharynx, larynx, not examined. Thymus absent. Esophagus and trachea negative.

"Lungs: Left lung smooth externally, marginal emphysema, crepitates throughout, floats in water, cut surface smooth, pale, and drips fluid. On the diaphragmatic surface is a caseated nodule 8 mm. in diameter. Right lung with the exception of the caseous nodule corresponds to the description of the left. Both lungs are quite free of pigment and weigh 2,870 grams. Peribronchial lymph glands are negative except for anthracosis.

"Heart: Cavities are quite empty, left ventricle contracted; aortic and pulmonary semilunar valves are competent, read by water test. Some fibrous thickening at the base of the aortic valves. One of the valves has small fenestration 3 mm. long. Pulmonary valves show no change. The mitral orifice admits three fingers, and the valve shows a small amount of fibrous thickening, especially near the free borders. The tricuspid orifice admits five fingers; the valves show no changes. The left ventricle has an aberrant corda tendina extending from the septum to the left wall. Ventricular wall measures 16 mm.; right ventricular wall measures 5 mm. Heart muscle is firm and very yellow, but not mottled. Heart weight, 300 grams. There is a slight increase of the subepicardial fat. There are a few atheromatous patches in the ascending aorta.

"Spleen: About one-half larger than normal; capsule is smooth, parenchyma is quite firm; malpighian bodies prominent; there is an evident increase of connective tissue. The organ weighs 225 grams.

"Kidneys: Right weighs 175 grams. Section pale, cortical markings not well seen; relation between cortex and medulla is normal; capsule strips readily; pelvis is normal. Left kidney weighs 135 grams. Answers to the description of its fellow.

"Ureters, bladder, testicles, and adrenals present no abnormal appearances.

"Liver: Extends 4 cm. below costal arch; capsule smooth and glistening—presents yellowish mottled appearance. Cut surface mottled yellow and red, lobules well seen. Liver cuts with decreased resistance. Weighs 1,600 grams. Bile ducts patent, bile dark brown, no concretions. Pancreas shows no changes.

"Stomach: Of normal size, externally is normal; mucosa is covered with much grayish-yellow mucus; no parasites.

"Small intestines: External appearance normal. Intestines contain a very large amount of very tenacious mucus. In the duodenum was found one hook worm. One hundred and fifty were found in the jejunum and upper part of ileum, being most numerous in the upper and middle jejunum. None found in last 18 inches of ileum. They were very adherent, and were in the proportion of about four females to one male. At point of attachment of some of the parasites was found a small ecchymotic spot, and scattered along the rest of the mucosa a few other ecchymotic spots were seen—possibly points of previous attachment. The mucus in places was blood-stained. Mesenteric glands were enlarged and of a pinkish color. Appendix lies in false pelvis to the outside of psoas muscle and behind cecum—bound down by fibrous adhesions and curled at its tip.

"Spinal cord shows no microscopic changes.

"Bone marrow removed from femur. Marrow is yellow and very fatty. At two points it had a reddish color, but was even here practically all fat.

"*Anatomic diagnosis:* 1. *Uncinaria duodenalis* of small intestine. 2. Ecchymosis of intestinal mucosa, and hemorrhage into intestinal lumen. 3. General anemia and edema of dependent portions of body. 4. Bilateral hydrothorax. 5. Hydropericardium. 6. Edema of lungs and caseous tuberculosis of lower left lobe. 7. Slight atheroma of aorta. 8. Fatty degeneration of heart and liver. 9. Aberrant corda tendina. 10. Passive congestion and fatty degeneration of liver. 11. Chronic interstitial splenitis."

WESTERN STATES.

MISSOURI.

St. Louis, 1893 1 case, 0 death.

BLICKHAHN's (1893a) case; probably imported from Germany and caused by *Agchylostoma duodenale*. See STILES (1902b, p. 208).

St. Louis, 1901 1 case, 0 death.

DYER's (1901) case. See STILES (1902b, p. 213).

CALIFORNIA.

San Francisco, 1902 2 cases, 0 death.

Letters from Drs. G. H. EVANS and MARY HALTON, 1902: Two soldiers who returned from the Philippines. Through the kindness of the observers I was able to examine specimens from one of these cases, and to convince myself that they belonged to the American species. The previous history of the patient was not obtained.

San Francisco, 1903 2 cases, 0 death.

BROWN (1903, p. 107): Three patients infected with *Strongyloides*; 2 of these (natives of Porto Rico) were also infected with *Uncinaria*.

? **Locality** 3 cases, 0 death.

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Public Health and Marine-Hospital Service of the United States.

WALTER WYMAN, Surgeon-General.

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February, 1903.

AN EXPERIMENTAL INVESTIGATION
OF TRYPANOSOMA LEWISI.

BY

EDWARD FRANCIS.



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AN EXPERIMENTAL INVESTIGATION OF TRYPANOSOMA LEWISI.

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The great importance of a thorough familiarity with trypanosomes is seen when we consider that within the past year and a half the recognition of trypanosomes was forced upon our governmental medical officers in the Philippines, where the army horses and mules were dying in great numbers, due to the presence in their blood of a parasite which the untrained eye might readily regard as a spirillum or a filaria, but which proved to be the trypanosome of Surra (1). It is probably best in the beginning to say something of trypanosomes in general. They are animal parasites (Hematozoá) of large size which are often found in enormous numbers free in the blood plasma, but do not invade the interior of the blood corpuscles. They manifest a strikingly vigorous eel-like motility as they dart among the corpuscles, pushing them aside.

The *Trypanosoma Evansi* is the cause of surra, a fatal disease of horses and mules in India and the Philippines.

The *Trypanosoma Brucii* causes the tsetse fly disease, or Nagana, which attacks the horses and cattle in central Africa.

The *Trypanosoma equinum* is the cause of mal de caderas, a disease of horses, etc., in South America.

Dourine, or *maladie du Coût*, a disease of horses and dogs, particularly in Algeria and Spain, has been attributed to the *Trypanosoma equiperdum*.

The *Trypanosoma Lewisi* is the cause of a nonfatal affection of wild rats, which harbor spontaneously the parasites in enormous numbers in their blood plasma.

And now within the past year we have a trypanosome of man.

Whether the six trypanosomes mentioned above represent really distinct species or whether two or more of them will be found to be identical must remain for further scientific investigation. Morphologically all six have many structures in common, but they also show

certain differences. Still, an accurate differential diagnosis on the basis of structure alone is not altogether practicable.

As a harmless inhabitant of the blood still other trypanosomes are harbored by the hamster, certain fish, frogs, and birds.

OCCURRENCE AND MORPHOLOGY OF *TRYPANOSOMA LEWISI*.

Our studies were made entirely with the rat trypanosome (*Trypanosoma Lewisi*). The natural host is the wild gray rat and the sewer rat. These rats harbor the trypanosomes in their blood and they infect each other spontaneously.

Crookshank (2) investigated a number of rats in London and found that 25 per cent of the apparently healthy rats harbored trypanosomes.

Rabinowitsch and Kempner (3) examined the blood of fifty wild rats caught about the city of Berlin and found that eighteen of them were infected with trypanosomes.

Laveran and Mesnil (4) found two cases of infection among forty-three sewer rats examined in Paris.

We examined sixty of our home rats caught in different parts of Washington and found no cases of infection.

Natural infection of the white or spotted rats has never yet been found, although they are very susceptible to the infection by inoculation. We inoculated the trypanosome into white rats and examined the blood in fresh and stained preparations.

The parasite may be considered in three parts: The body, the undulating membrane, and the flagellum. The body is the elongated portion which has attached to its side the undulating membrane and to one of its ends the flagellum. The body of the adult parasite measures 13 to 25 micra in length, which is about two to three times the diameter of a red blood corpuscle. Its breadth is 2 to 4 micra. The body runs to a beak-like process at one end, while at the other end there is a free flagellum 7 to 15 micra in length. The flagellate end is considered the anterior or front end because locomotion takes place in the direction of the flagellum, while the beak end is designated the hind end, since it follows the body in locomotion. In the hinder fourth of the body, about 4 micra from the end, there is a short rod-shaped structure, the long axis of which is at a right angle to the long axis of the body; this spot is called the centrosome. It can with difficulty be seen in a fresh preparation. In the anterior fourth of the body of the parasite there is a larger strongly refractile spot, which is regarded as the nucleus.

The undulating membrane is a clear, fine, transparent membrane which presents an attached border, a free border, and a web. It takes its rise from the centrosome, and is fixed by its attached border along the length of the body of the parasite and terminates at the front end, while the outer free margin of the membrane is thickened into a rod-like border which is continued past the front end into the long free

flagellum. Thus the thick border of the undulating membrane is continuous behind with the centrosome and in front with the flagellum. The web of the membrane is difficult to stain and is seen only faintly in stained preparations. In a fresh preparation the body, the undulating membrane, and the flagellum appear to consist of a homogeneous, strongly refractile protoplasmic substance possessing marked contractility.

A disputed point is the biological relation of centrosome to nucleus. Rabinowitsch and Kempner, after a study of numerous stained and unstained preparations, especially of the developmental forms of the trypanosomes, and after an investigation of the views held in regard to the nucleus of the flagellata, came to the conclusion that the centrosome and nucleus are an interdependent whole which corresponds to the nucleus of the other flagellata. They maintain that in the early stage of development of the parasite the centrosome and nucleus represent a whole which, as the parasite becomes older, breaks into two parts which pass to either end of the adult parasite. They consider the trypanosome's nucleus as made up of two parts more or less separated; the small oval spot situated in the hind end of the trypanosome they designate the nucleolus, while the larger structure found in the front end they call the chromatin heap.

Wasielewski and Senn (5) think that the centrosome is in no way whatever connected with the nucleus. They consider the trypanosome as made up of two parts, the "plasma" and the "periplast." The plasma is the body of the parasite containing the nucleus. The periplast is the outer covering of the trypanosome and embraces the centrosome, undulating membrane, flagellum, and an outer coat investing the body of the parasite. According to this view the centrosome is intimately connected with the undulating membrane. It is the root from which the thickened free border of the undulating membrane springs and has nothing to do with a nucleolus, as Rabinowitsch and Kempner point out, nor with a micro-nucleus, as held by Plimmer and Bradford. In their illustrations the outer coat of the parasite takes a different stain from the body plasma.

Laveran and Mesnil mention that blood kept some time shows trypanosomes reduced to centrosome and flagellum, and that the continuity of the centrosome and flagellum can be seen. We have seen the same thing in stained preparations of fresh blood.

CYCLE OF DEVELOPMENT.

We kept fresh blood in hanging drop at room temperature and at 37° C. and watched it for hours without seeing any of the parasites advance in development beyond the stage which they presented when the blood was first drawn. The different stages of development can be seen only with the help of animal experiments. It is very doubtful if multiplication of the organisms occurs outside the body.

Rabinowitsch and Kempner were the first to employ white rats inoculated intraperitoneally for the study of development, and they were able to trace the entire cycle of development by the examination at short intervals of the blood of a white rat so infected. By a similar examination of a great many rats we were able to get all the forms of development.

Multiplication by transverse division.—The first thing to be observed is a change in the outlines of the trypanosome. Its sharp beak becomes blunt; the flagellate end is no longer sharp, but rounded; the slender body becomes thickened and swollen; both the longitudinal and the transverse diameters of the parasite are increased. There is a multiplication of nuclei and centrosomes to a number which seldom exceeds five each, and they are arranged in a line parallel to the long axis of the parent trypanosome. After the division of nuclei and centrosomes, the next step is the formation of the new flagella, each of which takes its origin in a centrosome and generally emerges from the parent trypanosome on the side which bears the undulating membrane (Pl. III, fig. 9). These new flagella gradually attain full length. The old flagellum becomes destroyed and disappears. The cleavage of the protoplasm of the parent occurs along lines at right angles to the long axis, so that each new segment of protoplasm is equipped with a nucleus, a centrosome, and a flagellum.

The daughter cells are soon free in the blood and are readily distinguished from the adults by their small size and oval outline. They, however, gradually lengthen out into the familiar picture of the adult.

Multiplication by longitudinal division.—This form of multiplication has much in common with the transverse division. There is the same change in outline of the parent and the same multiplication of centrosomes and nuclei to a number of from two to six. The arrangement of the latter, instead of being in a line parallel to the long axis of the parent, is in a transverse line. The new flagella next appear. They arise at the front end of the parent and are arranged closely about the old flagellum and correspond in number to the centrosomes. (Pl. I, fig. 3.) Rabinowitsch and Kempner, in speaking of the origin of the new flagella, mention that in the flagellata the formation of the new flagella takes place so rapidly that the investigators can not follow the process accurately in all its details. They do not hold to the view that flagella multiply by a cleavage in the long axis of the old flagellum. It is their opinion that the new flagella arise from the body plasma and their principal support for such an origin is that in transverse division the new formation of flagella does not occur at the end close about the old flagellum, but on the side at a considerable distance away from the old flagellum.

Multiplication by segmentation.—These three forms of division are separated only for description. They may all be seen side by side in the same preparation and in some instances the distinction between them is not altogether clear, especially between the longitudinal and

transverse modes. Division by segmentation begins with a curving of the parasite which continues until the two ends approach each other and finally meet, thus giving the parasite a globular form. The flagellum becomes lost and the undulating membrane disappears. The nuclear multiplication now occurs and proceeds in the same manner as in the longitudinal and transverse division. The nucleus becomes swollen, appears coarsely granular, and divides into two parts, each of which probably divides and subdivides.

In stained preparations Rabinowitsch and Kempner saw small bodies in the new nuclei. These bodies, which were about the size of centrosomes, took the stain deeply and were placed at the periphery of the nucleus. Whether centrosomes can arise from nuclei or only from other centrosomes is a point of controversy and is mentioned under morphology.

Whatever may be the source of the centrosomes their number always keeps pace with the number of nuclei. No matter how many are formed there is a formation of a corresponding number of centrosomes until in the end there are as many centrosomes as nuclei in the trypanosome.

As regards the manner of the arrangement of nuclei and centrosomes in the globular parent, we find them in two concentric circles. The outer circle contains the nuclei and the inner circle the centrosomes. In the cleavage of the protoplasm there are as many segments as number of nuclei. While in transverse and longitudinal division there are not more than 6 daughter cells, we find in this form as many as 16. Plate III, figure 12, shows 14 segments.

Cleavage proceeds from the periphery to the center of the mass, so that a rosette arises as in the segmentation of malaria. Each segment of protoplasm contains a centrosome and a nucleus, and a flagellum develops from each. In hanging drop these rosettes are seen to be in agitation as soon as they acquire their flagella. Finally the segmentation is complete and the daughter trypanosomes are seen as small oval young forms free in the blood. Rabinowitsch and Kempner, in speaking of segmentation, refer to the hanging together of the young by their sharpened ends and their gradual separation from each other; but they maintain that the young parasites may develop into adults before the radial arrangement is broken up, as is shown in their figure 27 (Rabinowitsch and Kempner, 1899, pl. 3, fig. 27.) We have not seen them develop into adults while so arranged. The rosettes seem to become dissolved while their members are still young. We should think that figure 27 represented an autoagglutination of adults rather than an advanced stage of a rosette, for the reason that the members are evidently adults instead of young forms.

THE DURATION OF THE INFECTION.

Rabinowitsch and Kempner found that artificially infected white rats harbor these parasites in general from four to six weeks. They

saw them disappear from the blood in some cases in from one to two weeks, sometimes earlier. Two rats retained their parasites three to four months.

Wasielewski and Senn found trypanosomes five and one-half months after injection in several instances and no animal lost his parasites before six weeks.

Jurgens (6) found that his animals kept their blood parasites as a rule one to two months, seldom later. In two cases numerous parasites were found after seven months. Only once did he find the infection to last shorter than one month.

We found the duration of the infection to be seven to fourteen days. The long periods of infection of three to six months, or even of six weeks, we have not seen. The trypanosomes never returned spontaneously to the rats after they had once become free of them. We were unable to find any wild rats infected spontaneously, but the duration of such an infection is, according to Rabinowitsch and Kempner, much longer than an artificial infection of white rats. They state that in an infection of the wild rats the parasite, so far as they have observed, did not disappear from the blood.

MOTION OF THE TRYPANOSOME.

If a thin film of fresh blood is watched beneath a cover slip on a slide the parasites are seen to be in striking activity, darting among the corpuscles and pushing them aside, but never entering the corpuscles or engulfing them. The motion is too violent to be closely followed by the eye, so in order to gain an accurate perception of the mechanism of the motion the film must be very thin, so that the trypanosome will be subjected to the pressure between the cover and slide, and consequently his motion becomes very slow.

We see that the parasite generally moves with the flagellum (anterior end) in front, but if he meets an obstruction he withdraws for only a short distance in the direction of his blunt (posterior) end, and then resumes in his original direction. The movement takes place by means of the undulating membrane and the flagellum. When in motion the body of the parasite rotates on its longitudinal axis, thus moving in a screw-like manner. This causes the undulating membrane to appear as if it were spirally arranged around the organism. The waves, starting in the flagellum and traveling along the undulating membrane, are plainly seen. Motion will persist in a cover glass preparation for several hours.

SYMPTOMS IN RATS.

Rabinowitsch and Kempner mention loss of appetite, slight loss of weight and debility, but say that none died from the infection. They regard the disease in white rats as mild compared with the same in wild rats.

Jurgens reports severe sickness and death.

Many of our rats died. Plain evidences of sickness coupled with heavy blood infection were almost sure to give a fatal result. We made no pathological study of the internal organs.

STAINING.

In the beginning we attempted to stain the parasites with the ordinary aniline dyes. We used eosin, methylene blue, hemalum, carbo-fuchsin, and carbo-thionin, but all were very disappointing. We then turned to the Romanowsky double stain of methylene blue and eosin, Goldhorn's polychrome methylene blue and Jenner's stain, all of which give most beautiful effects indeed; perhaps the best is Romanowsky's stain. Some modification of this stain is the one which many of the investigators have employed within the last few years for trypanosomes. In the hands of the various experimenters this stain seems to give somewhat different effects. The two points on which they all agree are that the nucleus takes a beautiful rose red and that the protoplasm takes a blue color. Rabinowitsch and Kempner state, and it is clearly shown in their figures, that in the hind part of the adult parasite there is an oval or round spot of a homogeneous structure which takes the stain uniformly and intensely red. In the front end of the trypanosome is a larger body with a net-like structure which stains rose red. In their hands the protoplasm of the body and the free outer border of the undulating membrane and the flagellum stained quite uniformly blue. The web of the membrane remained unstained.

Wasielewski and Senn state that the protoplasm is almost homogeneous, stains a light blue, and has a fine granular structure. In it are one to three clear, oval areas, generally in front of the centrosome, which can be considered as vacuoles. With the Romanowsky stain they succeeded in giving a red tint, not only to the centrosome and nucleus, but to the free border of the undulating membrane, to the web of the undulating membrane, to the flagellum, and to the entire border of the body of the parasite. They state, however, that the tint of the centrosome, flagellum, undulating membrane, and "periplast" was a bluish red.

Laveran and Mesnil picture the centrosome and body of the parasite as blue, and the nucleus, flagellum, and undulating membrane as red. With Romanowsky's stain we gave a blue color to the body of the trypanosome. The nucleus took a rose red, and the centrosome was deep red. The flagellum and border of the undulating membrane were red, and the web of the undulating membrane was faintly red.

Wright's modification of Romanowsky's stain.—Wright (7), in speaking of Romanowsky's stain, which came out in 1891 as a differential stain for the chromatin and cytoplasm of the malarial parasite, refers to the difficulties and uncertainties attending the preparation of the stain until it became finally modified by Leishman (8), whose

method Wright has further simplified. Wright, in his directions for preparing the stain, says to add 1 per cent methylene blue to a one-half per cent solution of sodium bicarbonate and steam the mixture in an Arnold steam sterilizer for one hour, which renders the methylene blue polychromatic. When cold he adds eosin until the color changes from blue to purple and a metallic scum forms on the surface and a black precipitate appears in suspension. The precipitate is collected on a filter, dried, and dissolved in methyl alcohol. Wright gives the following summary for using the stain:

1. Make films of blood, spread thinly, and allow them to dry in the air.
2. Cover the preparation with the alcoholic solution of the dye for one minute.
3. Add to the alcoholic solution of the dye on the preparation sufficient water, drop by drop, until the mixture becomes semitranslucent and a yellowish metallic scum forms on the surface. Allow this mixture to remain on the preparation for two or three minutes.
4. Wash in water, preferably in distilled water, until the film has a yellowish or pinkish tint in its thinner or better spread portions.
5. Dry between filter paper and mount in balsam.

Goldhorn's stain (9).—Dry the film and fix in pure methyl alcohol fifteen seconds, wash in running water, stain in 0.1 per cent aqueous solution of eosin for thirty seconds, wash in running water, stain one minute in Goldhorn's polychrome methylene blue, wash in water, dry in air, mount in balsam.

The polychrome methylene blue is made as follows:

1. Dissolve 2 grams of methylene blue and 4 grams of lithium carbonate in 300 c. c. of warm water.
2. Heat in porcelain dish in a boiling water bath fifteen minutes.
3. Pour into a glass-stoppered bottle and set aside for several days.
4. Render only slightly alkaline by adding 4 to 5 per cent acetic acid solution.

With this stain we have obtained beautiful preparations showing the chromatin of the ring form of æstivo-autumnal malaria and the chromatin of the tertian parasite. We have also well-stained preparations of blood platelets. It is a good stain for the nuclei of animal parasites. It shows the chromatin of the segmenting bodies of malaria, the chromatin of the crescents, the eosinophilic and neutrophilic granules and nuclei of leucocytes. Mast cells and myelocytes are well stained.

Jenner's stain.—This stain is not so good for trypanosomes as the other two. Equal parts of 1 per cent aqueous solutions of eosin and methylene blue are mixed and set aside for twenty-four hours. The mixture is filtered, the precipitate is washed with water and dried and then dissolved in methyl alcohol. In using this stain no previous fixing is necessary. After staining one to three minutes the specimen is thoroughly washed until the corpuscles appear pink. Dry in the air and mount in balsam.

The stain can be bought ready for use from dealers, or a powder

can be gotten from Grüber, which is to be dissolved in methyl alcohol.

We have gone at some length into these stains, for without some one of them the investigator will attain little in the way of getting instructive preparations of trypanosomes and because their more general use may bring out some points still unmentioned in the structure of animal parasites generally. Since a thorough understanding of the stains is necessary to fine work, the reader is referred to the original articles mentioned in the bibliography.

ACTIVE IMMUNITY.

With very few exceptions a single infection with trypanosomes renders the rats free from parasites thereafter. Rabinowitsch and Kempner had no second infections following the injection of heavily infected trypanosome blood into the peritoneal cavity of rats, which, after artificial inoculation, had become spontaneously free of parasites. Laveran and Mesnil, in their series of thirty, found two susceptible to a second infection. One of our rats infected by feeding and another infected by intrastomachal injection proved susceptible to a second infection by intraperitoneal inoculation in two and five months, respectively, after they had become free of parasites. Their second infections lasted only three days.

PASSIVE IMMUNITY.

From our knowledge of the antitoxins of the bacterial diseases, we are led naturally to the investigation of the protective property of the serum of immunized rats, and it is found that there is produced a specific immune serum. The serum of rats which have been immunized by one or more inoculations of trypanosome blood does give protection to other rats within certain limits. Laveran and Mesnil found that their most active serum came from a rat which had been given 13 inoculations.

If we add in vitro 1 c. c. of immune serum to 1 c. c. of trypanosome blood and inject the mixture into a fresh rat, no infection will follow. We also separated the two injections in time to see whether immune serum would prevent infection if injected before the trypanosome blood; likewise, whether the immune serum would prevent infection if it were injected after the injection of infected blood. Our results were somewhat variable, but in general they corresponded to the limits of time which have been set by Rabinowitsch and Kempner and confirmed by Laveran and Mesnil, namely, that 1 c. c. of immune serum injected into a fresh rat twenty-four hours before or twenty-four hours after the injection of trypanosomes will prevent infection. They found that emulsions of the spleen, bone marrow, liver, or brain conferred no passive immunity.

The limits of the preventive and curative power of the immune

serum, although quite narrow, are, however, as wide as we would expect.

Five pregnant rats were inoculated, with a view to finding whether their young would acquire an immunity by placental transmission. Although the mothers bore a very heavy infection, we could never demonstrate any parasites in the fetuses nor did the young show any evidences of an increased resistance to subsequent infection. Laveran and Mesnil mention one immune rat which had two litters; the first litter was immune, the second susceptible. In this connection we may mention that no case of placental transmission of malaria has been reported in which the possibility of postnatal infection has been excluded.

SUSCEPTIBILITY OF ANIMALS TO *TRYPANOSOMA LEWISI*.

The wild rat and the sewer rat are the only animals in which there is a spontaneous conveyance to each other. The white and spotted rats are susceptible by inoculation only. Young rats are more susceptible than old ones. Rabinowitsch and Kempner report the failure to infect pregnant rats. We inoculated five pregnant females, and all bore heavy infections. No one has found that the white rats harbored trypanosomes spontaneously in their blood. No investigators have succeeded in infecting other animals with rat trypanosomes except Laveran and Mesnil, who infected guinea pigs. We tried in vain to infect guinea pigs, rabbits, white mice, cats, a dog, a goat, and a horse by intraperitoneal inoculation. In one guinea pig we found six parasites in the blood twenty-four hours after inoculation, but subsequent examinations of the blood showed an absence of all parasites, so that this can not be considered a case of infection. Laveran and Mesnil, after intraperitoneal injection of a guinea pig with 1 c. c. of blood rich in trypanosomes, found multiplication forms in the peritoneum two to five days after injection. They had numerous failures in bringing about a blood infection, but some guinea pigs showed parasites in the blood in the proportions of 1:20 and 1:50 of the red blood corpuscles. The infection was of short duration.

When attempting to infect the various animals other than rats we injected large amounts of heavily infected rat blood. In some cases we injected a rat's entire blood. White rats and wild rats are so susceptible that only one to three drops of infected blood, mixed with a little sterile salt solution or bouillon and injected intraperitoneally, will cause a marked infection.

AUTO-AGGLUTINATION.

We have brought forward the use of this term to signify the agglutination of a rat's own trypanosomes while still circulating in his own blood. If daily examinations are made of the blood of an infected rat,

the parasites will be seen to show agglutination during the period of their decline in numbers and disappearance from the blood. When auto-agglutination is well advanced we see very few parasites occurring singly. They are collected into masses; but these masses in turn show a tendency to collect close together, which must necessarily leave certain drops of blood almost free from all parasites while other drops will show typical fields of auto-agglutination. On this account a single hanging drop or a single stained preparation taken from a rat is not sufficient to give a correct idea of the condition of the parasites in the blood. As many as eight to ten slides may be made in which will be found only a few scattered parasites, and the next slide will show pictures such as are seen in Plate II, figure 8, and Plate IV, figures 13 and 14. Figure 13 represents a single focus of agglutination, and in figure 14 there are three such foci near to each other.

A close examination of these agglutinations will show that they have nothing to do with the rosettes of multiplication. They are found in the blood after the period of multiplication has passed, and they are made up of adult parasites instead of young forms. We consider these agglutinations as an evidence of the presence of agglutinin in the rat's blood and as an omen of an impending rapid disappearance of the parasites from the blood. We have in the laboratory in several instances seen the agglutination of the parasites in a rat's blood for a few successive days before a sudden disappearance of all parasites over night. We conclude from this observation that agglutination is a step toward dissolution, and that it foretells the disappearance of the trypanosomes from the blood.

From a daily study of the blood of numerous cases in which agglutinations of parasites were present we were able to prophesy that active immunity was near at hand.

It is not every case of infection which shows auto-agglutination. We would explain its absence on the ground of an insufficient generation of agglutinin. As will be referred to later, there is a difference in the agglutinating power of the immune sera of different rats. As soon as a rat loses his parasites he is considered an immune and his serum is admitted to have agglutinating power on fresh parasites to which it may be added. The agglutinating power of the immune serum is, moreover, attributed to the agglutinin which it contains. Now, we think that the production of this agglutinin is a gradual process which is begun before a rat loses his parasites, and we think that just previous to the disappearance of all parasites from a rat's blood there is a short period during which he may generate sufficient agglutinin to agglutinate the parasites circulating in his own blood. We would offer as a possible explanation of our own cases that perhaps our trypanosomes were more virulent than those used by other observers. This view seems to be supported by the shorter time which elapsed between inoculation and heavy blood infection, the

more rapid disappearance of the parasites from the blood, and the greater number of deaths among the white rats.

The agglutinated parasites have a most orderly arrangement in the shape of a rosette, with their posterior ends close around a central point and their flagella at the periphery. Each parasite has its own fine undulating motion.

AGGLUTINATION.

1. *By immune serum.*—Immunity and agglutinating power go hand in hand. As in late years agglutination has been proven for so many kinds of bacteria by their specific sera, so trypanosomes are found to respond in a somewhat similar way to immune serum.

Laveran and Mesnil probably made the most complete study of agglutination. The trypanosome blood which they used was subjected to defibrination, which of course left the corpuscles and the trypanosomes in the serum. We have found that the substitution of clotting for defibrination will show the agglutination to much better advantage in the hanging drop, since there will be no corpuscles in the field to obscure agglutination. The immune blood and the trypanosome-bearing blood may be drawn up into separate fine capillaries and allowed to coagulate. The clot is then drawn out at one end of the tube, leaving the clear immune serum behind in the one case and the clear serum containing trypanosomes in the other case. Now the dilutions can be made just as in the Widal reaction. We have, however, taken another precaution which Laveran and Mesnil did not observe. We removed all agglutinin from the trypanosome serum before starting the tests. We were led to this by the study of auto-agglutination.

If we should select for our agglutination tests blood in which auto-agglutination was already noticeable, we would immediately fall into the error of getting agglutination with ever so great a dilution of the immune serum, because the trypanosomes were agglutinated before we began. Again, if we selected blood in which there was no auto-agglutination, but in which there was considerable agglutinin, but still not enough to produce an auto-agglutination, we would still be in error if we attempted to determine the agglutinating power of an immune serum by testing it on trypanosome-bearing serum which was just on the verge of auto-agglutination. We must therefore take into consideration one element which does not enter an agglutination test on bacteria. The bacterial pure culture has a fixed, uniform composition, and until we are able to grow trypanosomes in pure culture on artificial media we will have to consider the element of agglutinin in the serum which contains the parasites.

Our plan was to draw the trypanosome blood from the rat, allow it to coagulate, draw off the serum containing the parasites, and dilute it with plain distilled water and filter it through a porcelain filter

under the influence of a vacuum. After four or five washings with large amounts of water, we let it filter until there remained behind a volume of fluid which equaled the original amount of serum. In this residue were the trypanosomes free from agglutinin. Dilutions were then made of the immune serum, and it was tested on the washed parasites. Some immune sera will not agglutinate in a dilution greater than 1:1. An agglutinating power of 1:5 or 1:10 is common. One of our rats showed typical agglutination in a dilution of 1:200.

It is interesting to watch in hanging drop an agglutination by a weak serum. At first two or three parasites are seen, joined by their posterior ends. Others come up toward the center of agglutination, recede for some distance, and later join the others. Some disengage themselves from the rosette and then rejoin it, until finally a well-arranged rosette is formed. Two small rosettes will gradually approach each other and then unite to form one mass, which in turn is joined by others of smaller or larger size.

A remarkable fact is that the agglutinated parasites do not lose their motility. There is not the diminution of motility before agglutination that is seen in a typhoid reaction, and while agglutinated each parasite retains a regular vibration. In agglutination with a strong serum the process takes place rapidly. The parasites rush together in great numbers, and the masses may be of macroscopic size. If a strong specific serum is used, the agglutinated masses are very compact and the individual parasites are tightly drawn together, so that there is little motion. In a general way the parasites are all pointed toward a center, but still they overlap and cross each other very much. With a weak serum the parasites are held together in a loose manner, permitting of more individual movement to each organism and more orderly arrangement, and there are fewer parasites to each rosette.

We often found agglutination almost complete, in which case very few parasites were to be seen free in the field. It may be only partial. The parasites may remain agglutinated until their death, or, if the serum is weak, a disagglutination may follow. Specific sera exposed to 55° C. for thirty minutes did not lose the agglutinating power, but a temperature of 65° C. maintained for half an hour destroyed its activity.

Laveran and Mesnil found that trypanosomes killed by chloroform or formalin were agglutinated by the same sera which agglutinate the living, but the parasites have no orderly arrangement in the mass. It is a remarkable fact that Rabinowitsch and Kempner found that "the trypanosome serum shows in no way whatever the property of agglutination."

2. *By normal sera.*—The action of normal sera in dilution of 1:1 was tested on the trypanosomes. The cat and horse sera were strongly agglutinating. The goat and rabbit sera were feebly agglu-

tinating. The sera of the white rat, white mouse, and guinea pig were negative.

Laveran and Mesnil state that the normal sera of the chicken and horse caused complete agglutination. The sera of the sheep, dog, and rabbit produced a partial reaction, while the pigeon, frog, guinea pig, white and spotted rats, and sewer rats caused none whatever. They also state that, although the blood of chicken and horse have agglutinating power, they do not protect against infection.

3. *In the ice chest.*—If trypanosome blood is drawn from the rat under aseptic conditions and sealed in pipettes and placed in the ice box, the parasites will usually join into beautiful rosettes after twenty-four hours. We found them agglutinated in blood which had been eighty-three days in the ice chest.

TRANSMISSION OF THE DISEASE.

1. *By intraperitoneal inoculation.*—Although white and spotted rats have never yet been found to harbor trypanosomes spontaneously in their blood, we find in them a very susceptible host for experimental inoculations, and it was with them that most of our work was done. Comparative studies show that a heavy blood infection is obtained by intraperitoneal injection sooner than by any other form of inoculation. The blood to be injected is mixed with 1 c. c. of saline solution or bouillon and injected with a hypodermic syringe. The period which elapses between the intraperitoneal inoculation and the first appearance of the parasites in the blood is variable, depending upon the amount of trypanosomes injected and the stage of development of the injected parasites.

Rabinowitsch and Kempner place the time at three to seven days, although they observed parasites in a few instances within the first day.

Laveran and Mesnil give three to seven days as the average time before the blood infection. They found a few in the circulation after five or six hours.

Jourgens found that the first presence of parasites in the tail blood occurred three or four days after inoculation, seldom later. After inoculation with 1 c. c. of blood he found them in tail blood in several instances after twenty minutes.

The parasites appeared in the tail blood in our cases usually on the second day. This was chiefly because we injected as a rule larger doses and used heavily infected blood in which were many developmental forms.

There has been considerable discussion as to where the principal seat of multiplication takes place in intraperitoneal inoculation. Rabinowitsch and Kempner regard the peritoneal fluid as a better nutritive medium for the development of the parasites than the blood and think that the chief seat of development and multiplication is in the peritoneum. In one to five days after intraperitoneal injection

of trypanosome blood they found in the peritoneum several engaged in development. They think that as soon as development is perfected in the peritoneum the parasite disappears from the peritoneal fluid into the blood. They give some weight to the fact that in one case of intravenous injection of parasites multiplication did not occur until the fifth day. Laveran and Mesnil state that there is less multiplication in the blood than in the peritoneum.

Our observations speak for the blood as the principal seat of development. On examination of the peritoneal fluid at varying times after injection we did not find division forms, nor did we find the first existence of parasites in the tail blood accompanied by division forms. The most reasonable explanation seems to be that if a large amount (0.75 c. c.) of heavily infected blood be injected into the peritoneal cavity, the small young forms and the long slender adults pass at once by the lymphatics into the general blood stream in sufficient numbers to be detected in cover-slip preparations within twenty-four hours or even within twenty-minutes, but the rosettes and other large division forms which are injected into the peritoneal cavity are prevented by their size from passage through the lymph channels and remain behind in the peritoneal cavity until their division is complete, when the young then pass through the lymph passages into the blood, leaving the peritoneum permanently free from parasites.

Daily examination of the blood for one to two days after the first appearance of parasites in the tail blood shows at most only a very gradual increase in the number of parasites present, but suddenly there comes an enormous swarming of the blood with parasites, and the presence of rosettes and other division forms indicate that multiplication is going on in the blood. The slide from which the microphotographs (Pl. III, figs. 11, 12) were made shows at least two dozen rosettes and was taken from the rat on the second day after the first appearance of parasites in the tail blood. It is not unusual to find parasites in the proportion of 1:2 red blood cells. Exceptional instances are met with in which the blood corpuscles are outnumbered by the trypanosomes. The duration of the period of multiplication is often no more than twelve to twenty-four hours. By the fourth day after the first entrance of parasites into the blood the height of infection has been reached. Rabinowitsch and Kempner found no division forms in the blood on the fourth day after the first appearance of parasites in the tail blood.

2. *By subcutaneous inoculation.*—Blood infection occurs by this form of injection a little later than by intraperitoneal inoculation. The shortest time in our cases between inoculation and the appearance of parasites in the blood was three days. Multiplication proceeded in the blood until it swarmed with myriads of parasites. This would seem to be additional evidence to the superiority of the blood over the peritoneal fluid as a nutritive medium for the development of the parasites, for in cases where the parasites had advantage of

the peritoneal fluid in addition to the blood their number did not reach a point beyond the number obtained by subcutaneous injection.

3. *By intrastomachal injection.*—We have read some discussion bearing on the natural mode of infection with trypanosomes in which infection followed the eating of a trypanosome rat by a healthy one. It was held that this could not be considered a case of intrastomachal infection because the possibility of the entrance of the parasites through wounds about the mouth, lips, or teeth had not been excluded.

We therefore arranged a series of experiments in which we thought all likelihood of infection through wounds was removed and that infection occurred through the stomach. Twelve white rats were chloroformed sufficiently to prevent any struggling. Then a small-sized catheter well oiled was passed into the stomach without encountering any resistance; injection of trypanosomes was made through the catheter, and the rats were then placed in separate cages and examined daily for parasites in the tail blood. Eleven out of the 12 developed blood infections fully as heavy as was obtained by any other form of inoculation. The time which elapsed before their appearance in the blood was as follows: Two in four days, one in five days, three in six days, three in seven days, and two in eight days.

We see that infection is considerably delayed by this method, the earliest being in four days and the latest in eight days.

4. *Transmission by feeding.*—The success of the intrastomachal injections naturally lead to a series of experiments to determine whether infection would not occur by feeding when all precautions were taken to prevent any wounding about the teeth or mouth.

Seven white rats, apparently free from mouth wounds, were put into separate cages to prevent fighting, and they were fed with soft food, so that no wounds would result from the gnawing of bones. They were each given a single feeding with the entire blood of a trypanosome rat. No other part of the infected rats was given to them. Trypanosomes appeared in the tail blood in five of the seven at periods of time which averaged six days.

Wild rats were then subjected to similar feeding experiments. In the blood of five wild rats we found parasites after three, seven, eight, nine, and ten days. Some of the rats had enormous numbers in their blood, while others had comparatively few.

We conclude from these experiments that infection may take place through the digestive tract and that the spread of the disease among wild rats may be due to feeding upon one another, especially since the instinct of fighting and pluck is so well implanted in them and is brought into action on slight provocation. We found it necessary to separate the wild rats in our stock cage to prevent losses from injuries inflicted on the weaker ones by the stronger.

After beginning the feeding experiments we were surprised to read the results obtained by Rabinowitsch and Kempner. They made intrastomachal injections through a stomach tube in four rats, but were

unsuccessful, although they repeatedly introduced into one rat blood rich in parasites and nourished one rat chiefly by this material. They placed together an infected tame rat and a noninfected wild rat, and state that after a hot combat the tame one was killed by the wild rat and eaten with much pleasure. Parasites appeared in his blood after ten days. They did not regard this as a case of infection necessarily by the digestive tract, as the parasites may have entered by the numerous bites and wounds, nor do we regard it as such; but we think we have in our experiments removed the likelihood of entrance through wounds and have established the existence of infection through the digestive tract alone.

5. *Transmission by fleas*.—Rabinowitsch and Kempner, with a view to finding the natural mode of transmission, placed together an infected white rat with a healthy white rat. The latter became infected in eleven days. They repeated the experiment and found parasites in the blood of another rat after fifteen days. A gray rat showed parasites in his blood after fifteen days' confinement with two infected animals. The three rats which became infected had many fleas on them. Examination of a great number of teased preparations of the fleas did not reveal any of the trypanosomes in them. They then mashed up fleas collected from infected rats and injected this material into the peritoneal cavity of nine white rats. Five became infected. Likewise four rats were injected intraperitoneally with mashed-up lice, but no infection followed. The next experiment was to determine whether the bites of fleas were infective. Twenty fleas were collected from infected rats and placed on one healthy white rat, which after three weeks time was found to be infected with trypanosomes. They say that from this one positive experiment they conclude that fleas can carry trypanosomes, and in the absence of proof of another way of conveyance they are of the opinion that fleas are the ordinary medium of infection.

Jourgens states that his experiments were not then complete, but that he had kept infected rats and healthy rats together in the same cage without infection taking place, although the animals were strongly beset with fleas, while a later inoculation of the sound animals proved them to be susceptible.

6. *Trypanosomes in lice*.—Laveran and Mesnil found trypanosomes in the stomachs of lice which infested infected rats, but do not report a conveyance of the disease by lice bites.

7. *Trypanosomes in Stegomyia fasciata*.—We may be pardoned for mentioning a subject which is entirely outside of rat trypanosomes—but it may have some future bearing on the disease—to state that within a year Durham (10) has reported finding trypanosomes in a mosquito; thus adding one more to the rapidly growing list of diseases through which this little creature threatens the public health. Durham's report is inserted.

A small bat (*Phyllostoma*) which could not be examined at once was placed in a gauze cage, and with it a specimen of *Stegomyia fasciata*. The next day the bat

was dead and the mosquito full of fresh blood. This blood contained abundant trypanosomes, whose shape is quite different from the usual ones found in rats, Nagana, etc. * * * Although flagellates, coccidia-like bodies, etc., often were found from time to time in the 80 mosquitoes which were dissected, this was the only time that trypanosomes were found.

THE PARASITES OUTSIDE THE BODY.

We must confirm the reports of Wasielewski and Senn, and of Rabinowitsch and Kempner, that if hanging drops are made of blood containing parasites in different stages of division a careful watching of the specimen will not witness the completion of the division process, whether the specimens be kept at room temperature or in the ice box or in the incubator.

Jurgens found quite the contrary. He made three hanging drops of the same blood with the same platinum loop. No. 1 was placed at 37° C. No. 2 was kept at room temperature. No. 3 was immediately dried, fixed, and stained, and examination showed it to contain parasites preparing to divide. On the next day Nos. 1 and 2 were dried, fixed, and stained. The stained specimen of No. 2 always showed the same conditions as No. 3; but in the stained specimen of No. 1 there were rosettes and division forms, none of which was seen in No. 3. Therefore, he concludes that it is possible for certain stages of the parasite under certain conditions to increase outside the body.

It will require further investigation to determine whether the parasite exists in the flea's body in the forms known to us as young forms, division forms, and adult parasites, or whether there is still another form which has not yet been described. The fact of not being able to find in infective fleas any form resembling a trypanosome has a close parallel in what occurred in one of our rats. A white rat in whose blood we saw many parasites in agglutination for several days suddenly showed an absence of parasites from the blood. We immediately performed an autopsy and made a careful search of the lungs, liver, spleen, kidneys, heart, brain, and bone marrow for parasites, but could find none. We were struck by the amount of granular debris in the liver and kidneys, but could not see any trypanosomes in them.

An emulsion was made of the kidneys and injected into the peritoneal cavity of a young rat. The liver was treated in the same manner and injected into two young rats. On the ninth day all three rats had parasites in their blood and a few days later developed heavy infections. While some unknown form of the parasite may have been present, it is of course possible that well-known forms of the trypanosomes were in the kidneys and liver and escaped notice.

Jurgens produced infection with 0.000005 c. c. of blood. If this amount of blood be added to 1 or 2 c. c. of salt solution, the dilution of the parasites would be so great that they might readily be missed in hanging-drop preparations.

The possibility of the existence of some very minute form of the

parasite was perhaps excluded by our filtration experiments. Trypanosome blood was diluted with fifteen parts of physiological salt solution and subjected to a porcelain filter under the influence of a vacuum. The filtrate was found to be noninfective to rats.

In blood kept in the ice chest for eighty-one days we found living trypanosomes. Their motion was of a trembling, vibrating character. Very few crossed the field. Many were arranged in rosettes. In the stained preparations we found parasites in which the positions of the centrosome and nucleus were reversed, the centrosome being anterior to the nucleus. In many of the trypanosomes there was granular degeneration, and there was also much free granular debris, which probably represented the remains of degenerated parasites. At room temperature and at 37° C. the parasites are short-lived. The different investigators found them capable of producing an infection of rats after being kept in the incubator and at room temperature from four to seven days. The parasites when kept at 12° to 16° C. in sterile pipettes can maintain their vitality very much longer than under any other condition.

Laveran and Mesnil kept parasites alive in defibrinated blood or in equal parts of defibrinated blood and physiological salt solution for forty-seven days in the ice box. At the end of this time the blood was injected into rats and produced a blood infection in nine days. In blood kept under the same conditions for fifty-one days they saw no parasites by microscopic examination, but it was infective for rats.

Jurgens found only a few parasites in blood kept in the ice chest for fifty-three days, but rats injected with it showed parasites in their tail blood after seven days.

Bacteria have a very detrimental effect on the life of the parasites outside the body. In hanging drops of trypanosome blood which has become infected with bacteria the parasites rapidly die. Blood drawn for keeping in the ice box must be kept under sterile conditions. Trypanosomes are killed after an exposure of a few minutes at 55° C. Wasielewski and Senn found living trypanosomes in the bloody urine of a rat. Rabinowitsch and Kempner were never able to find the parasites in the feces or urine.

THE TRYPANOSOME OF THE HAMSTER.

This animal harbors a parasite which has been studied by Rabinowitsch and Kempner. They find that it resembles the rat trypanosome in that it can hardly be differentiated from it morphologically and has the same process of development. On the other hand, they could not convey it to rats, and some of the rats which were refractory to hamster trypanosomes later proved susceptible to rat trypanosomes.

These facts, taken together with the fact that they could not convey rat trypanosomes to the hamster, led them to conclude that the hamster trypanosome and the rat parasite represent two different physiological varieties, which morphologically are almost inseparable.

TRYPANOSOMIASIS IN MAN.

Trypanosomiasis as a disease of man has not yet become acclimatized in the United States. At least no cases have been reported in this country. There have appeared in the foreign journals within the past year, however, eleven authentic instances of infection with trypanosomes.

A case of trypanosomiasis in a European, by Dutton (11) and Forde (12).—The patient was a European, 42 years of age, master of a government steamer on the Gambia River, in West Africa.

On May 10, 1901, the patient was admitted into the hospital at Bathurst, West Africa, suffering from what was regarded as malarial fever. Examination of his blood did not show malarial parasites, but there were seen extremely active bodies which were regarded as filaria. Three weeks later the patient was invalided to Liverpool, but returned in December, 1901, to Bathurst, where Dr. Dutton, of the Liverpool School of Tropical Medicine, examined the patient's blood and found the same parasite which Forde had probably seen seven months previously and which he at once recognized as a trypanosome.

The symptoms were an irregularly intermittent fever, a very marked erythema multiforme of the trunk and limbs, an oedematous condition of the face beneath the eyes and of the ankles, an acceleration of respiration and pulse rates, debility and loss of flesh, and enlarged spleen. The symptoms persisted throughout the eight months during which he was under observation and showed no reaction to treatment further than a slight abeyance under Fowler's solution.

Dutton found, while making examinations of fresh blood during the month of December, 1901, an average of one and one-half trypanosomes to each cover slip preparation. One preparation showed as many as 15 parasites. This case continued in its chronic course until the last week of life, during which week the disease assumed an acute type and the patient died January 1, 1903.

Dutton suggests the name *Trypanosoma gambiense* in case that further study shows it to be a new species.

A second case of trypanosomiasis in a European, by Manson (13).—The patient was the wife of a missionary on the Upper Kongo, where she had lived for a year. On account of sickness she returned to London.

Dr. Manson, of the London School of Tropical Medicine, recognizing the same group of symptoms which the patient of Dutton and Forde presented, made systematic, careful examinations of her blood daily for two weeks. During the two weeks no trypanosomes were found, but at the end of this time the parasites were readily seen in the peripheral circulation.

Trypanosomes in the blood of a West Africa native, by Dutton (14).—Three trypanosomes were found in a single smear from the

blood of a child 3 years of age, a native of Gambia. The child showed no symptoms of disease.

Eight additional cases of human trypanosomiasis.—In the British Medical Journal for February 7, 1903, there is a "preliminary account of the investigations of the Liverpool expedition to Senegambia," by Dutton, Annett, and Todd. They found trypanosomes in the blood of a white trader who had been twenty years in Gambia. The highest number found in a fresh preparation was seven. The patient had lost 45 pounds within the past year, complained of weakness and breathlessness, and had slight fever at times. The spleen was enlarged and there was pitting of the ankles. Four other cases of infection of natives were found.

Three more cases of human trypanosomiasis are reported in the British Medical Journal for March 28, 1903, by Dr. Patrick Manson. They came from the European community on the Kongo, as did also Dr. Manson's first case. A blotchy erythema and attacks of fever characterized these cases.

The finding of trypanosomes in man, associated with a well-defined group of signs and symptoms, is no small contribution to the disentanglement of the diseases of the Tropics. These cases will lead to the recognition of others, perhaps, in the tropical parts of our own continent or of Asia. The disease has been found in West Africa, and with this new fact in parasitology before us its geographical distribution may be found to have a much wider range. On account of the interest which surra claims in the Philippines and on account of the recognition within the past year of trypanosomes in man, and since the process of development and conveyance of these blood parasites have heretofore been little investigated in this country, we have undertaken this study.

We wish to call attention to the autoagglutination, transmission by feeding, transmission by intrastomachal injection, and to the staining.

I am glad to thank the director of the laboratory, Dr. M. J. Rosenau, for his interest in outlining the work.

I am indebted to Dr. John F. Anderson, the assistant director, for valuable suggestions, especially in the staining of the parasites.

I also desire to express my thanks to Dr. H. B. Parker for making the microphotographs.

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PLATE I.

Drawn with Abbe drawing camera. Stained according to Romanowsky method.
× 1,000.

Figs. 1 and 2. Adult trypanosomes.

Fig. 3. Parasite undergoing longitudinal division.

Fig. 4. Transverse division.



FIG. 1.



FIG. 2.

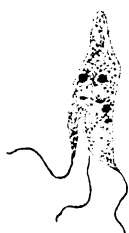


FIG. 3.



FIG. 4.

PLATE II.

Fig. 5. Beginning multiplication.

Fig. 6. Same, later stage. The mother parasite is still visible.

Fig. 7. A multiplication rosette.

Fig. 8. Auto-agglutination.



FIG. 5.



FIG. 6.

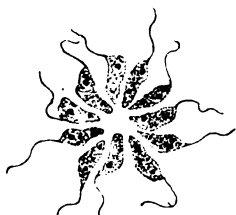


FIG. 7.

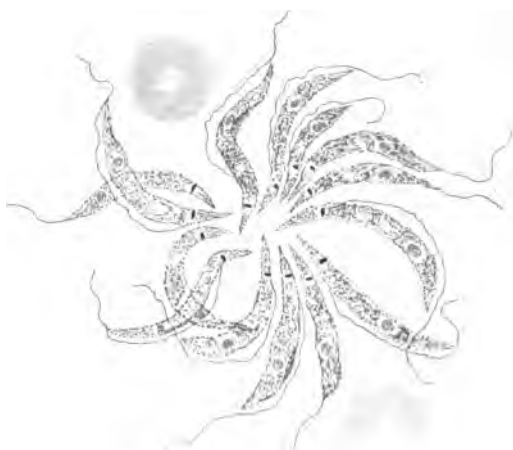


FIG. 8.

PLATE III.

Microphotographs. Stained with Goldhorn's stain.

- Fig. 9. Transverse division. The two new flagella coming off from the side which bears the undulating membrane.
- Fig. 10. Advanced stage of division. The parent parasite is curved around a red-blood corpuscle.
- Figs. 11 and 12. Multiplication by segmentation. The rosettes are composed of 9 and 14 daughter parasites.

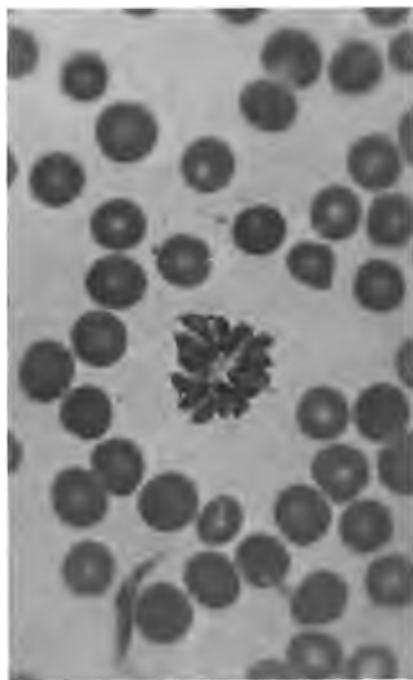
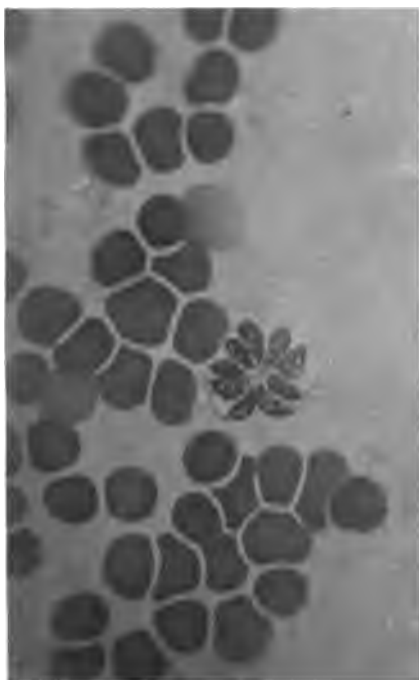
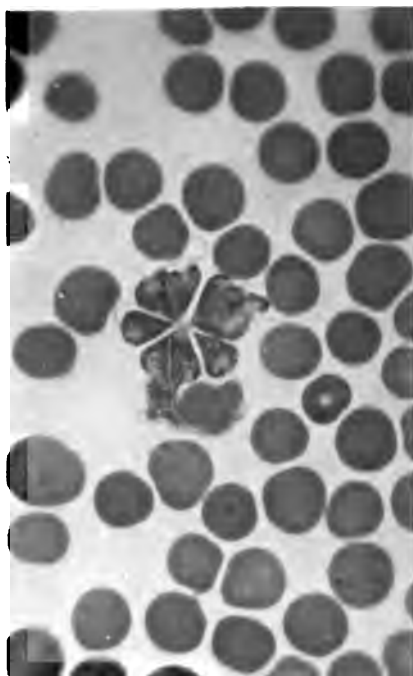
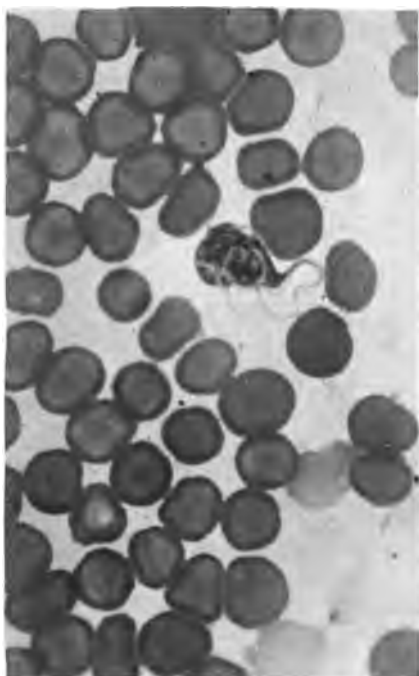


PLATE IV.

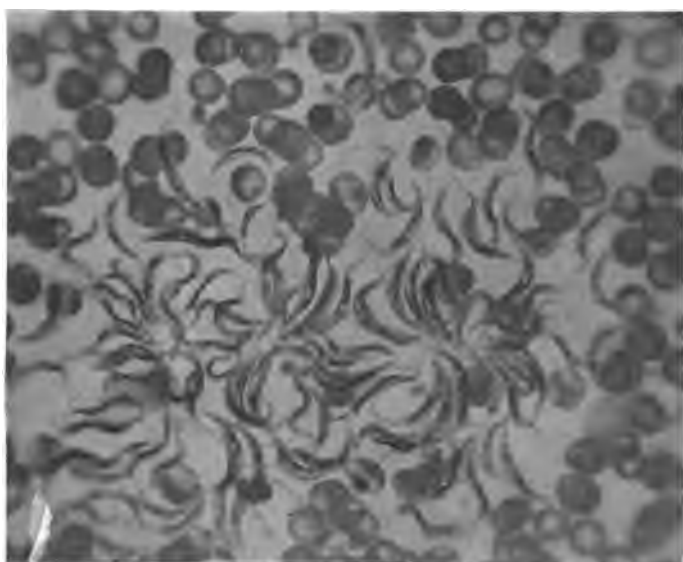
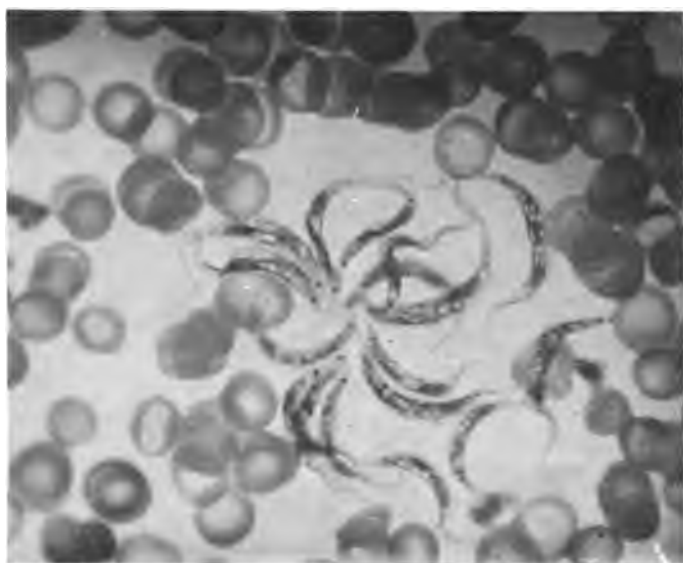
Auto-agglutination.

Fig. 13. Shows a single focus of agglutination.

Fig. 14. Shows three such foci.

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TREASURY DEPARTMENT.
Public Health and Marine-Hospital Service of the United States.
WALTER WYMAN, Surgeon-General.

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THE BACTERIOLOGICAL IMPURITIES
OF
VACCINE VIRUS.

AN EXPERIMENTAL STUDY.

BY
M. J. ROSENAU,
DIRECTOR OF HYGIENIC LABORATORY.



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1903.

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THE BACTERIOLOGICAL IMPURITIES OF VACCINE VIRUS. AN EXPERIMENTAL STUDY.

(By MILTON J. ROSENAU, Director of the Hygienic Laboratory, Passed Assistant Surgeon, U. S. Public Health and Marine-Hospital Service.)

OBJECT OF THE WORK.

The widespread prevalence of smallpox in our country during the past few years gave abundant opportunity to corroborate the great prophylactic value of vaccination. Hundreds of thousands of vaccinations have been performed during this period with very satisfactory and beneficial results. However, we were not surprised to hear of some accidents and complications. There were sore arms and a few fatalities resulting from wound complications.

The question immediately arose whether the cause of these complications was contained in the vaccinal matter or whether it resulted from subsequent contamination of the wound from outside sources.

Clinical studies can not be depended upon to settle this question, because infected "takes" may result from other causes than a contamination of the virus.

To determine this question, therefore, we made bacteriological studies of the number and kinds of micro-organisms that contaminate the different makes of vaccine virus found on the open market. In our studies special attention was given to the pus cocci and to tetanus.

We were rather surprised in visiting some of the vaccine farms to find that the operators permitted a certain degree of carelessness in preparing and collecting material for the glycerinated virus, trusting, as they stated, "to the glycerin to take out the impurities." This opinion of the exalted power of glycerin we have shown to be based upon error. Glycerin will destroy a certain number of bacterial impurities, but it is impotent against gross contamination or spores. Our studies of the germicidal power of glycerin will be made the subject of another paper.

All the vaccines examined were purchased in the open market from reliable pharmacists, who keep the virus under suitable conditions of light and temperature. Our results therefore represent the impurities found in the vaccine virus sold to the practitioner.

INEVITABLE IMPURITIES IN BOVINE VACCINE VIRUS.

Vaccinia is a specific disease, the cause of which has not been determined. We are, therefore, working somewhat in the dark. We are compelled to vaccinate our patients with a virus containing micro-organisms other than those causing vaccinia. The importance of using a virus as pure as possible need not be emphasized here, for we do not want to inoculate our patients with any other infection than the one which protects the individual against smallpox. On account of this danger human virus has been discarded in many countries, despite the fact that human virus is believed to be superior to bovine virus so far as the reliability and the duration of its immunizing power are concerned.

The production of bovine virus by propagating it from heifer to heifer is credited to Negri, of Naples, about 1842. It took some years for the advantages of this virus to be appreciated. Practically no other kind is now used in the large communities of Europe and in our own country. The great advantage of bovine virus, in addition to the ease with which it may be procured, is that it absolutely eliminates the possibility of the transmission of syphilis and other infections peculiar to the human family.

Now, although bovine virus is free from the danger of conveying the infectious diseases peculiar to man, it is liable to other equally undesirable contaminations. For instance, in addition to the micro-organisms that are specific for vaccinia, it contains the pus cocci and the bacteria that live normally upon and in the skin of the calf, and these micro-organisms always contaminate bovine virus. It is impossible to use germicidal agents in the treatment of the vaccinal eruption of calves, as such substances would kill the potency of the virus. Therefore, it is evident that even the greatest care will not insure such virus against "foreign" organisms.

OBJECT OF GLYCERINATING THE VACCINE PULP.

In order to eliminate the danger of the contaminating bacteria, Dr. Moncton Copeman, in 1891, devised the method of mixing the pulp with sterile glycerin of first quality. The advantages which Copeman claimed for the glycerin were that it not only prevented the growth and multiplication of the bacteria, always found in bovine virus, but gradually destroyed those which were present.

Glycerin can hardly be dignified with a place among the germicides, although the object of adding it to vaccine virus is to destroy the contaminating micro-organisms. Bacteria are slowly killed by glycerin, just as they are killed by drying; the glycerin is supposed to produce its bactericidal effects by a process of slow dehydration. Germs with thick envelopes resist it indefinitely. It has no action upon endogenous spores at all; in fact, it is a preservative of such infections as

tetanus, malignant edema, anthrax, and the like. In 80 and 90 per cent glycerin, we have kept as common and as easily destroyed an organism as the golden pus coccus alive forty-one days; the colon bacillus lived seventy days in 60 per cent glycerin; anthrax spores remained alive and virulent two hundred and seventy-eight days in glycerin. This work will soon be made the subject of another report.

Glycerin, therefore, can not take the place of care and cleanliness in the preparation of vaccine virus.

The effect of mixing glycerin with the virus is to gradually destroy both the bacteria and the vaccine, but fortunately the ordinary pus cocci and nonsporulating bacteria generally succumb before the viability of the vaccine organism is destroyed, and therefore there is an interval when the glycerinated virus will still cause a typical "take," but will contain comparatively few foreign micro-organisms. It is evident that if the glycerinated virus is used before this interval it has no advantage over the dry point, and if used after this interval it is inert. Therefore, from a theoretical view point glycerinated virus should be freer from impurities if used just at the right time. Manufacturers state that they usually glycerinate the virus from four to six weeks before putting it on the market.

The dry points, on the contrary, may be sold as soon as made and, if kept in a cool place protected from the light, they probably remain viable a longer time than the glycerinated virus under similar conditions. It is well known that pus cocci and the other bacteria which frequently contaminate vaccine virus die quickly when dry. These same bacteria, however, live a comparatively long time in dry vaccine virus, probably on account of the protection of the albuminous matter in which they are imbedded.

The glycerinated virus is made from the "pulp" that is scraped from the site of the eruption. This pulp consists of the inflammatory exudate and macerated cuticle. The mass is intimately ground with 50 to 60 per cent of glycerin, then placed aside in a cool place for several weeks until the pyogenic cocci and other pathogenic organisms disappear.

Until recently the dry points were always prepared by using the serum or "lymph" which exudes from the site of the eruption, after the crusts and epithelial layers have for the most part been removed. The sterile ivory points are then coated with this serum by means of a brush or the points are dipped directly into the exudate. This lymph is largely blood serum, mixed with inflammatory exudate. At present the dry points are mostly prepared from the glycerinated pulp. That is to say, the glycerinated virus, after it has stood a sufficient length of time to free itself of bacterial impurities, is dried upon ivory points. Sometimes the glycerinated-virus is mixed with sterile blood serum in order to facilitate its drying and adhesion to the ivory point.

SEASONAL VARIATION IN THE PURITY OF VACCINE VIRUS.

A glance at the following tables will show the unevenness of the product of some manufactures. It will be noted that the contaminating microbes seemed to rise in numbers during the winter of 1901-2, when the demand for vaccine virus was very great, indicating perhaps haste in its production, or the marketing of an unripe product. It will also be noted that there is a marked improvement in the glycerinated product this winter (1902) as compared with that placed on the market last year. We believe that our warning, showing the feeble germicidal properties of glycerin, and an exposition of the fact that some of the glycerinated virus found upon the market was "green," helped materially toward this beneficial result. (See article in American Medicine, April 19, 1902, page 637, "Dry points versus glycerinated virus from a bacteriologic standpoint," read at a meeting of the New York Academy of Medicine, February 20, 1902, by M. J. Rosenau.)

Tables comparing the number of bacteria in the dry points and glycerinated virus marketed by the various manufacturers at different times.

MANUFACTURER NO. 1.

Dry points.			Glycerinated virus.		
Winter, 1901-2.	April and May, 1902.	November and December, 1902.	Winter, 1901-2.	April and May, 1902.	November and December, 1902.
4,923	7,240	0	192	22	1
6,240	7,326	0	1,332	24	2
9,050	7,625	1	1,456	28	2
9,289	7,625	1	6,249	46	2
9,884	9,430	2	6,876	72	3
10,629	10,050	3	7,274	101	3
14,826	10,340	3		—	4
20,828	10,345	3		48	4
	12,075	5		80	5
	12,100	11		102	5
				110	9
				112	
				194	

MANUFACTURER NO. 3.

847	2,518	7,465	111	3,011	41
906	2,727	8,702	138	4,309	70
2,088	3,275	10,022	246	6,022	87
2,750	3,866	10,265	352	6,670	95
6,528	4,359	10,982	1,121	7,294	112
13,030	4,446	12,923	1,750	7,427	152
	5,577	15,552	2,070	9,309	230
	8,048	25,574	2,263	11,169	234
	8,384	26,411	2,440		239
	19,107	31,380			

Tables comparing the number of bacteria in the dry points, etc.—Continued.

MANUFACTURER NO. 4.

Dry points.			Glycerinated virus.		
Winter, 1901-2.	April and May, 1902.	November and December, 1902.	Winter, 1901-2.	April and May, 1902.	November and December, 1902.
1,530	636	243	1,414	24	3
2,160	1,998	262	1,540	54	4
2,376	2,069	420	1,842	68	6
8,024	2,433	424	2,928	84	11
9,688	2,726	452	10,372	97	16
12,800	2,780	465	11,232	144	19
	3,448	476	18,404	153	31
	3,960	479		165	37
	5,402	526		259	77
	8,160	576		393	113

MANUFACTURER NO. 5.

13	1	604	84	6	2
18	5	810	86	24	2
20	6	1,088	97	28	2
27	7	1,156	127	55	2
110	9	1,199	160	105	2
182	10	1,204	170		3
219	10	1,399	245		3
220	10	1,452	257		4
297	50	2,246	768		5
458		2,584	1,680		6
575			1,700		
648			2,069		
7,200			10,400		
14,200			17,000		
15,760					

MANUFACTURER NO. 6.

None examined.			369	21	0
			747	694	0
			1,592	1,877	0
			1,912	2,368	1
			2,106	2,689	2
			2,578	3,326	2
			3,819	4,208	4
				4,352	4
				8,094	5
					11

Tables comparing the number of bacteria in the dry points, etc.—Continued.

MANUFACTURER NO. 7.

Dry points.			Glycerinated virus.		
Winter 1901-2.	April and May, 1902.	November and December, 1902.	Winter 1901-2.	April and May, 1902.	November and December, 1902.
	63	3	116	28	4
	88	3	160	32	4
	95	5	1,600	35	5
	106	5	2,400	35	5
	120	6	8,000	37	6
	125	7		44	6
	128	21		47	7
	164	31		51	7
	342	48		59	9
	380	67		101	9

MANUFACTURER NO. 8.

450	2,548	853	2,100	13	8
476	3,405	868	2,200	14	10
516	3,805	887		20	11
3,325	3,829	1,800		21	12
3,475	4,026	1,847		21	13
3,600	4,321	1,946		21	14
	4,445	2,074		26	16
	4,665	2,244		28	16
	5,121	2,912		31	19
	5,355	3,754		42	20

MANUFACTURER NO. 9.

	195	5,952	11,840	12	7
	245	7,682	12,350	32	10
	312	7,560	12,960	116	13
	440	7,980	15,680	214	25
	496	8,748	16,480	236	43
	576	8,748	17,680	345	47
	578	9,744	17,920	370	70
	665	11,020	23,800	415	81
	805	12,972	28,640	416	85
	1,000	19,872	30,080	541	105

MANUFACTURER NO. 10.

			0	0	7
			0	0	14
			0	3	15
			0	3	17
			0	3	21
			0	4	22
			0	4	22
			0	5	31
			3	7	31
			4	9	53
None examined.					

TECHNIQUE EMPLOYED IN COUNTING BACTERIA IN VACCINE VIRUS.

The virus was suspended in a measured quantity of sterile bouillon and agitated so that all the clumps were broken up and a uniform suspension obtained as nearly as possible. The dry points were first softened in a small quantity of bouillon and then rubbed clean, always using the usual bacteriological precautions to prevent contamination from the outside. The glycerinated virus was mixed with a small quantity of bouillon, and the capillary tube washed out by drawing the liquid in and out of the tube a number of times.

The mixing was done in test glasses of appropriate size and the mixture thoroughly agitated. It is very important to agitate the suspension thoroughly in order to break up the clumps. This may best be done by drawing the fluid into pipettes and blowing it out vigorously, repeating the operation a number of times.

This suspension was now planted in agar and plated on Petri dishes. No less than three plates were made of each point or capillary tube; one or two drops of the suspension being planted in the first plate, five or ten drops in the second, and the total quantity remaining in the third plate. In this way the figures gave an accurate count of all the colonies that grew from each vaccine examined, excepting in those instances where the vaccine contained excessive numbers of bacteria. In such cases it was necessary to estimate the total number from the first or second plate.

The plates were grown in the incubator at 37° C., and the counts made when the maximum growth appeared, usually upon the third day.

It is practically impossible to determine the absolute number of organisms in a given virus, for it consists of an inflammatory product which is very variable in its physical characteristics. Upon dry points it coagulates into a hard, insoluble film; and mixed with glycerin it always contains little masses and agglutinated flakes and particles which hold enmeshed the micro-organisms. It is practically impossible to ultimately break up these masses. Therefore, the suspensions are not uniform and the counts we make are only approximations. Micro-organisms have a well-known tendency to group or cling together, so that every colony upon an agar plate does not represent the growth from one single microbe. The figures as given, therefore, are misleading only in that they give an underestimate of the number of organisms contaminating vaccine virus, and therefore some of the results, as bad as they are, do not fully represent the actual conditions.

The capillary tubes containing glycerinated virus vary considerably in capacity; some hold three and four times as much as others. This fact partly explains the discordance in some of the figures, but it is not sufficient to justify the marked discrepancy which we have found existing between tubes from the same package and bearing the same laboratory number as given by the manufacturer.

The following tables give the results of our work during the summer (April and December, 1902). We were of colonies, especially from repeated our work very carefully. Grubbs, Dr. Parker, Dr. Fra

NUMBER OF BACTERIA IN VACCINE MARKET, WITH A SPECIAL

The number of bacteria found is an important factor as the kind of virus cocci would be of the common saprophytes found to contaminate vaccine as well as of the care taken in the found in glycerinated virus is

We counted 190 dry points. The points averaged 4,354 bacteria

Some points contained as few as 100 bacteria. The highest count for glycerinated virus was 30,080. Some were sterile, i. e., gave no bacterial bouillon.

SERIES 1.

give the results of our studies during the win-

MANUFACTURER NO. 1.

Number of organisms found.	
Dry points.	Glycerinated virus.
a 9,289	b 192
a 14,826	b 1,332
a 20,828	b 1,456
c ———	b 6,249
6,240	b 6,876
9,884	b 7,274
c ———	
4,923	
9,050	
10,629	

a Orange, yellow, and white staphylococci were isolated from these points; not pathogenic for mice, rats, and guinea pigs.

b Yellow and white staphylococci were isolated from these tubes. They produced no effect upon mice, rats, and guinea pigs.

c These short lines separate samples bearing a different number as given by the manufacturer.

This virus evidently contains more bacteria than a good virus should. The ordinary cocci of suppuration were found in both the dry points and the glycerinated virus; and although these organisms when inoculated into laboratory animals gave no results, there is little indication that under favorable circumstances they might not be pathogenic for man. While the glycerin has reduced the average number of bacteria found in this virus, it still has pathogenic bacteria that it should not contain; and, so far as the numbers are concerned, the samples examined, with one exception (192), are far above the number allowed a good virus.

MANUFACTURER NO. 3.

Number of organisms found.	
Dry points.	Glycerinated virus.
847	2,263
906	1,750
2,088	2,070
2,750	2,440
6,528	111
13,030	138
	246
	352
	1,121

A great many molds common to the air and to hay, were found in this virus, indicating stable contamination.

MANUFACTURER NO. 4.

Number of organisms found.	
Dry points.	Glycerinated virus.
1,530	10,372
2,376	11,232
<u>2,160</u>	<u>18,404</u>
8,024	1,414
9,688	1,540
12,800	1,842
	2,928

Here we have a manufacturer who puts up both kinds of virus, but his glycerinated product contains more bacteria than his dry points.

MANUFACTURER NO. 5.

Number of organisms found.	
Dry points.	Glycerinated virus.
110	84
182	768
220	1,680
575	1,700
7,200	2,069
11,200	10,400
15,760	17,000
<i>a</i> 219	<i>a</i> 86
<i>a</i> 297	<i>a</i> 97
<i>a</i> 458	<i>a</i> 160
<i>a</i> 648	<i>a</i> 257
<i>b</i> 13	<i>b</i> 127
<i>b</i> 18	<i>b</i> 170
<i>b</i> 20	<i>b</i> 245
<i>b</i> 27	

a Taken from the same lot and examined three months later.

b Another sample.

The only comment that it seems necessary to make on the product of this manufacturer is its unevenness. While the average is fair, we found two capillary tubes of glycerinated virus to contain an excessive contamination. It may be that these tubes were placed upon the market before they were sufficiently glycerinated.

MANUFACTURER NO. 6.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined.	369
	747
	2,106
	1,592
	1,912
	2,578
	3,819

MANUFACTURER NO. 7.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined.	116
	160
	1,600
	2,400
	8,000

MANUFACTURER NO. 8.

Number of organisms found.	
Dry points.	Glycerinated virus.
450	^a 2,100
476	^a 2,200
516	
^b <u>3,325</u>	
3,475	
3,600	

^aThe ordinary pus cocci (white, yellow, and orange) were isolated from this virus, but when inoculated into animals gave negative result.

^bThis line separates samples bearing a different number as given by the manufacturer.

Nine weeks later other samples of the glycerinated virus out of the same box were counted and gave only 30 bacteria per tube. This would seem to indicate that the virus was "green" when placed upon the market, and that if the manufacturer had kept it a few weeks longer it would have been freer from contamination.

MANUFACTURER NO. 9.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined.	11,840
	12,350
	12,960
	15,680
	16,480
	17,680
	17,920
	23,800
	28,640
	30,080

MANUFACTURER NO. 10.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined.	0
	0
	0
	0
	0
	0
	0
	0
	3
	4

SUMMARY OF SERIES I.

Winter 1901-2.

Number of organisms found.		
Dry points.		Glycerinated tubes.
13	0	2,100
18	0	2,106
20	0	2,200
27	0	2,263
110	0	2,400
182	0	2,440
219	0	2,578
220	0	2,928
297	3	3,819
450	4	6,249
458	30	6,876
476	84	7,249
516	86	8,000
575	97	10,372
648	111	10,400
847	116	11,232
906	127	11,840
1,530	138	12,350
2,088	160	12,960
2,160	160	15,680
2,376	170	16,480
2,750	192	17,000
3,325	245	17,680
3,475	246	17,920
3,600	257	18,404
4,923	352	23,800
6,240	369	28,640
6,528	747	30,080
7,200	768	
8,024	1,121	
9,050	1,332	
9,289	1,414	
9,688	1,456	
9,884	1,540	
10,629	1,592	
11,200	1,600	
12,800	1,680	
13,030	1,700	
14,826	1,750	
15,760	1,842	
20,828	1,912	
	2,069	
	2,070	
Number points examined 41	Number tubes examined 71	
Total bacteria 197,185	Total bacteria 333,586	
Average per point 4,809	Average per tube 4,698	

SERIES II.

The following tables give the results of our studies during the spring of 1902:

MANUFACTURER NO. 1.

Number of organisms found.	
Dry points.	Glycerinated virus.
7,240	22
7,326	24
7,625	28
7,625	46
9,430	72
10,050	101
10,340	—
10,345	48
12,075	80
12,100	102
	110
	112
	194

The dry points were dated "to be exchanged after June 3, 1902," and were examined May 23, 1902. The plates contained many colonies of a yellow staphylococcus resembling pus organisms.

The glycerinated virus was dated by the maker "to be exchanged after April 25, 1902," and was examined April 26, 1902.

MANUFACTURER NO. 3.

Number of organisms found.	
Dry points.	Glycerinated virus.
2,518	3,011
2,727	4,309
3,275	6,022
3,866	6,670
4,359	7,294
4,446	7,427
5,577	9,309
8,048	11,169
8,384	
19,107	

The dry points were guaranteed by the manufacturer until June 7, 1902, and were examined April 30, 1902.

The glycerinated virus was guaranteed by the manufacturer until June 17, 1902, and was counted April 30, 1902.

MANUFACTURER NO. 4.

Number of organisms found.	
Dry points.	Glycerinated virus.
636	24
1,998	54
2,069	68
2,433	84
2,726	97
2,780	144
3,448	153
3,960	165
5,402	259
8,160	393

The dry points were labeled by the maker to be returned after May 28, 1902, and were counted May 14, 1902.

The glycerinated virus was labeled by the maker to be returned after June 17, 1902, and was examined May 15, 1902.

MANUFACTURER NO. 5.

Number of organisms found.	
Dry points.	Glycerinated virus.
1	6
5	24
6	28
7	55
9	105
10	
10	
10	
50	

The dry points were labeled by the maker to be returned after May 18, 1902, and were planted April 25, 1902.

The glycerinated virus was labeled to be returned after May 11, 1902, and was examined April 25, 1902.

MANUFACTURER NO. 6.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined.	21
	694
	1,877
	2,368
	2,689
	3,326
	4,208
	4,352
	8,094

The glycerinated virus was stated by the manufacturer to be good until June 4, 1902, and was examined April 30, 1902.

Many yellow and white colonies grew on the plates from this vaccine resembling pus cocci. The following table shows the result of our study of 12 of these organisms:

Table of staphylococci isolated from

No.	Grouping.	Relation to Grams.	Indol.	Growth in milk.
³ 10	Staphylococci	Stains	Negative	Very slight acid product.
³ 10	do	do	do	No change.
⁴ 5	do	do	do	Light yellow
⁴ 10	do	do	do	Light brown.
⁴ 0	do	do	do	Light yellow
⁵ 10	do	Negative	do	Acid production; precipitate of casein.
⁶ 10	do	Stains	do	No change.
⁷ 23	do	do	do	do
⁸ 5	do	do	do	do
⁹ 20	do	do	Very slight	Alkaline, brownish yellow precipitate.
¹⁰ 65	do	do	Negative	No change.
¹⁰ 65	do	do	do	Dirty light blue, with scum on surface.

glycerinated virus of manufacturer No. 6.

Growth in gelatin.	Growth in bouillon.	Growth on potato.	Growth in glucose bouillon.	Growth in lactose bouillon.
No liquefaction...	Diffused cloudiness.	White granular growth; scanty.	No fermentation; cloudy all through.	No fermentation; cloudy at bend of tube.
Slow liquefaction.	Slightly cloudy...	Heavy growth; yellowish green.	No fermentation; slightly cloudy at bend of tube.	Do.
No liquefaction...	...do	Slight yellow growth.	No fermentation; cloudy at bend of tube.	Do.
...dodo	No growth.....	No fermentation; very slight growth at bend of tube.	No fermentation; very slight growth at bend of tube.
...dodo	Heavy yellow growth all over potato.	No fermentation; precipitate at bend of tube.	No fermentation; precipitate at bend of tube.
...do	Cloudy	Heavy white growth spreading.	No fermentation; slightly cloudy at bend of tube.	No fermentation; slightly cloudy at bend of tube.
Very slow liquefaction.	...do	Yellowish white growth; very heavy.	No fermentation; very slight growth at bend of tube.	No fermentation; very slight growth at bend of tube.
No liquefaction...	...do	Invisible growth.	...do	Do.
...dodododo	Do.
...dodo	Abundant white growth, slowly spreading.	...do	Do.
Slow liquefaction.	Slightly cloudy...	Slow growth, yellow granular.	...do	Do.
No liquefaction...	Slightly cloudy; some precipitate.	No growth.....	...do	Do.

MANUFACTURER NO. 7.

Number of organisms found.	
Dry points.	Glycerinated virus.
63	28
88	32
95	35
106	35
120	37
125	44
128	47
164	51
342	59
380	101

The dry points were stated by the maker to be good until June 15, 1902, and were examined April 26, 1902.

The glycerinated virus was labeled by the manufacturer to be good until May 1, 1902, and was counted April 26, 1902.

MANUFACTURER NO. 8.

Number of organisms found.	
Dry points.	Glycerinated virus.
2,548	13
3,405	14
3,805	20
3,829	21
4,026	21
4,321	21
4,445	26
4,665	28
5,121	31
5,355	42

Counted May 15, 1902. The packages contained neither date nor laboratory number.

MANUFACTURER NO. 9.

Number of organisms found.	
Dry points.	Glycerinated virus.
195	12
245	32
312	116
440	214
496	236
576	345
578	370
665	415
805	416
1,000	541

The dry points were prepared, according to the maker's label, May 1, 1902, and were counted May 15, 1902.

The glycerinated virus was not to be used after July 1, 1902, according to the maker's statement, and was examined May 15, 1902.

MANUFACTURER NO. 10.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined	0
	0
	3
	3
	3
	4
	4
	5
	7
	9

The glycerinated virus was marked to be returned after August 1, 1902, and was counted May 16, 1902.

SUMMARY OF SERIES II.

April and May, 1902.

Number of organisms found.			
Dry points.		Glycerinated tubes.	
1	5,355	0	110
5	5,402	0	112
6	5,577	3	116
7	7,240	3	144
9	7,326	3	153
10	7,625	4	165
10	7,625	4	194
10	8,048	5	214
50	8,160	6	236
63	8,384	7	259
88	9,430	9	345
95	10,050	12	370
106	10,340	13	393
120	10,345	14	415
125	12,075	20	416
128	12,100	21	541
164	19,107	21	694
195		21	1,877
245		21	2,368
312		22	2,689
342		24	3,011
380		24	3,326
440		24	4,208
496		26	4,309
576		28	4,352
578		28	6,022
636		28	6,670
665		28	7,294
805		31	7,427
1,000		32	8,094
1,998		32	9,309
2,069		35	11,169
2,433		35	
2,518		37	
2,548		42	
2,726		44	
2,727		46	
2,780		47	
3,275		48	
3,405		51	
3,448		54	
3,805		55	
3,829		59	
3,866		68	
3,960		72	
4,026		80	
4,321		84	
4,359		97	
4,445		101	
4,446		101	
4,665		102	
5,121		105	
Number points examined..	69	Number tubes examined....	84
Total bacteria.....	238,626	Total bacteria.....	88,879
Average per point.....	3,458	Average per tube.....	1,058

SERIES III.

The following tables give the results of our studies made during the winter, November and December, 1902.

Note especially how vastly superior the glycerinated virus in this series is to Series I and II. Eighty-nine capillary tubes examined averaged only 29 bacteria per tube, whereas in Series II, made in April and May, 1902, the average was 1,058, and in Series I, made in the winter 1901-2, it was 4,698.

MANUFACTURER NO. 1.

Number of organisms found.	
Dry points.	Glycerinated virus.
0	1
0	2
1	2 (Coc. 3.)
1	2
2	3
3 (Coc. 1.)	3
3	4
3	4
5	5
11 (Coc. 2.)	9 (Coc. 4.)

These points were labeled by the manufacturer to be exchanged after November 23, 1902, and were counted November 21, 1902.

The glycerinated lymph was labeled good to December 10, 1902, and was counted November 24, 1902.

The following organisms, some of which resemble the pyogenic cocci, were isolated:

Coccus No. 1.—This staphylococcus was one of 3 colonies which grew on our agar plates planted from a dry point of manufacturer No. 1.

The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon is slightly clouded with some white precipitate; on agar, a grayish white moist glistening growth; on potato, white growth, slowly spreading; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 2.—This staphylococcus was one of 11 colonies which grew on our agar plates planted from a dry point of manufacturer No. 1.

The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon is clear, with a yellow precipitate; on agar a lemon yellow, moist, shiny growth;

on potato, a scant yellowish growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 3.—This staphylococcus was one of 2 colonies which grew on our agar plates planted from a tube of glycerinated virus of manufacturer No. 1.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk, a slight alkaline production; does not liquefy gelatine; bouillon is clear, some white precipitate; on agar, a heavy, glistening pink pigment growth; on potato, very scanty pink growth; in glucose bouillon, no fermentation, slight growth at bend of tube; in lactose bouillon, no fermentation, slight growth at bend of tube.

Coccus No. 4.—This staphylococcus was one of 9 colonies which grew on our agar plates planted from a tube of glycerinated virus of manufacturer No. 1.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk, no change, but a white precipitate; does not liquefy gelatine; in bouillon, clear, white precipitate; on agar, a scanty white, glistening growth; on potato, very scanty white growth; in glucose bouillon, no fermentation, slight growth at bend of tube; in lactose bouillon, no fermentation, slight growth at bend of tube.

MANUFACTURER NO. 3.

Number of organisms found.	
Dry points.	Glycerinated virus.
7,465	41
8,702 (Coc. 5.)	70
10,022	87
10,265	95 (Coc. 9.)
10,982	112 (Coc. 10.)
12,923 (Coc. 6.)	152
15,562	230
25,574	234
26,411 (Coc. 7.)	239
31,380 (Coc. 8.)	

The dry points were labeled to be good until December 7, 1902, and were planted for study November 8, 1902.

The glycerinated lymph was labeled good until December 17, 1902, and was planted November 25, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 5.—This staphylococcus was one of 8,702 colonies which grew on our agar plates planted from a dry point of manufacturer No. 3. The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk, slightly acid production; does not liquefy gelatine; bouillon very cloudy, white pellicle, and precipitation; on agar, grayish-white, shiny, moist growth; on potato, heavy yellowish-white growth all over potato; in glucose bouillon, slight fermentation, cloudy all through; in lactose bouillon, slight fermentation, cloudy all through.

Coccus No. 6.—This staphylococcus was one of 12,923 colonies which grew on our agar plates planted from a dry point of manufacturer No. 3. The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; slight acid produced in litmus milk; liquefies gelatine very slowly; bouillon is slightly cloudy, with some white precipitation; on agar, a grayish-white, shiny, moist growth;

on potato, a white, granular, spreading growth; in glucose bouillon, no fermentation, slightly cloudy at bend of tube; in lactose bouillon, no fermentation, slightly cloudy at bend of tube.

Coccus No. 7.—This staphylococcus was one of 26,411 colonies which grew on our agar plates planted from a dry point of manufacturer No. 3. The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; rapidly liquefies gelatine; bouillon is clouded with white precipitation; on agar there is a white, moist, shiny growth; on potato, a white, granular growth, nearly invisible; in glucose bouillon, no fermentation, but growth at bend of tube; in lactose bouillon, no fermentation, but growth at bend of tube.

Coccus No. 8.—This staphylococcus was one of 31,380 colonies which grew on our agar plates planted from a dry point of manufacturer No. 3. The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; liquefies gelatine very slowly; bouillon is slightly cloudy, with white precipitation; on agar, a yellowish-white, moist, shiny growth; on potato, white, granular growth nearly invisible; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 9.—This staphylococcus was one of 95 colonies which grew on our agar plates planted from a tube of manufacturer No. 3. The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; strong acid production in litmus milk; does not liquefy gelatine; bouillon is very cloudy, white precipitate; on agar, a grayish-white, moist, shiny, glistening growth; on potato, very heavy, white growth all over; in glucose bouillon, fermentation $1\frac{1}{2}$ inch, growth all through; in lactose bouillon, fermentation three-fourths inch, growth all through.

Coccus No. 10.—This staphylococcus was one of 112 colonies which grew on our agar plates planted from a tube of manufacturer No. 3. The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; in bouillon, clear yellowish pellicle and precipitate; on agar, yellow, dry, and granular growth; on potato, very scanty, yellow growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

MANUFACTURER NO. 4.

Number of organisms found.	
Dry points.	Glycerinated virus.
243	3
262	4
420	6
424	11 (Coc. 13, 14.)
452	16
465	19
475 (Coc. 11, 12.)	31
479	37
526	77 (Coc. 15.)
576	113

The label of this manufacturer stated that the dry points were good till November 28, 1902. They were planted for counting November 18, 1902.

The glycerinated virus was labeled to be good till January 4, 1903, and was planted for study November 28, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 11.—This staphylococcus was one of 475 colonies which grew on our agar plates planted from a dry point of manufacturer No. 4.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; strong acid production in litmus milk; rapidly liquifies gelatine; bouillon is cloudy, with some precipitate; on agar there is a whitish-yellow, moist, shiny growth; on potato, a yellow growth, spreading slowly; in glucose bouillon, no fermentation, but cloudy all through; in lactose bouillon, no fermentation, cloudy all through.

Coccus No. 12.—This staphylococcus was one of 475 colonies which grew on our agar plates planted from a dry point of manufacturer No. 4.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk, acid production, coagulation and casein precipitate; bouillon is slightly cloudy, white precipitate; on agar, whitish gray, moist, glistening growth; on potato, white granular growth; in glucose bouillon, no fermentation, growth all through; in lactose bouillon, no fermentation, growth all through.

Coccus No. 13.—This staphylococcus was one of 11 colonies which grew on our agar plates planted from a tube of manufacturer No. 4.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon slightly clouded, yellow precipitate; on agar, growth transparent, thin layer, no pigmentation; on potato, scanty yellow growth; in glucose bouillon, no fermentation. slight growth at bend of tube; in lactose bouillon, no fermentation, slight growth at bend of tube.

Coccus No. 14.—This staphylococcus was one of 11 colonies which grew on our agar plates planted from a tube of manufacturer No. 4.

The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon slightly cloudy, some white precipitate; on agar, a lemon-colored, moist, glistening growth; on potato, no growth; in glucose bouillon, no fermentation, but growth all through; in lactose bouillon, no fermentation, but growth all through.

Coccus No. 15.—This staphylococcus was one of 77 colonies which grew on our agar plates planted from a tube of manufacturer No. 4.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon slightly cloudy, with some precipitate; on agar, a slight granular, scanty growth, no pigmentation; on potato, no growth; in glucose bouillon, no fermentation, growth all through; in lactose bouillon, no fermentation, growth all through.

MANUFACTURER NO. 5.

Number of organisms found.	
Dry points.	Glycerinated virus.
604	2
810	2
1,088	2
1,156	2
1,199	2
1,204	3 (Coc. 16.)
1,399	3
1,452	4
2,246	5
2,584	6

These dry points were stated by the manufacturer to be good until December 4, 1902. They were planted for counting November 7, 1902. Glycerinated lymph was stated by the manufacturer to be good November 19, 1902, and was counted November 24, 1902.

Organisms resembling the pyogenic cocci were isolated:

This staphylococcus was one of 3 colonies which grew on our agar plates from a tube of manufacturer No. 5.

Stains by Gram's method; does not produce indol in a 24-hour culture in litmus milk; does not liquefy gelatine; bouillon culture shows a white precipitate; on agar, a whitish gray, moist, shiny growth; on glucose bouillon, no fermentation, growth at bend of tube; on lactose bouillon, no fermentation, growth at bend of tube.

MANUFACTURER NO. 6.

Number of organisms found.	
Dry points.	Glycerinated virus.
	0
	0
	0
	1
	2
	2
	4
	4
	5
	11 (Coc. 17.)

The glycerinated virus was dated August 8, 1902, and was planted December 3, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 17.—This staphylococcus was one of 11 colonies which grew on our agar plates, planted from a tube of manufacturer No. 6.

The staphylococcus stains by Gram's method. Does not produce indol in a 24-hour bouillon culture; produces acid, with yellow pellicle and precipitate in litmus milk; liquefies gelatine very slowly; bouillon clear, with some yellow precipitate; on agar, a heavy yellow-orange growth; on potato, scant yellowish growth; in glucose bouillon, no fermentation, slightly cloudy at bend of tube; in lactose bouillon, no fermentation, slightly cloudy at bend of tube.

MANUFACTURER NO. 7.

Number of organisms found.	
Dry points.	Glycerinated virus.
3	4 (Coc. 19.)
3	4
5	5
5	5
6	6
7	6
21	7
31	7
48	9 (Coc. 20.)
67 (Coc. 18.)	9 (Coc. 21.)

The points were labeled to be good till December 15, 1902, and were planted November 24, 1902.

The glycerinated virus was labeled good till December 15, 1902, and was counted November 22, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 18.—This staphylococcus was one of 67 colonies which grew on our agar plates planted from a dry point of manufacturer No. 7.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon cloudy with white precipitate; on agar, whitish transparent, slightly granular, scanty growth; on potato, brownish white granular growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 19.—This staphylococcus was one of 4 colonies which grew on our agar plates planted from a tube of manufacturer No. 7.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; liquefies gelatine; bouillon cloudy, white pellicle and precipitate; on agar, white shiny growth; on potato, white granular growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 20.—This staphylococcus was one of 9 colonies which grew on our agar plates planted from a tube of manufacturer No. 7.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon clear with slightly white precipitate; on agar, whitish gray isolated colonies; on potato, no growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 21.—This staphylococcus was one of 9 colonies which grew on our agar plates planted from a tube of manufacturer No. 7.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; a slight acid production in litmus milk; slowly liquefies gelatine; bouillon cloudy with some white precipitation; on agar, heavy yellow growth; on potato, very heavy yellow granular growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

MANUFACTURER NO. 8.

Number of organisms found.	
Dry points.	Glycerinated virus.
853 (Coc. 22.)	8
868	10
887 (Coc. 23.)	11
1,800	12
1,847	13
1,946	14
2,074	16 (Coc. 25.)
2,244	16
2,912	19
3,754 (Coc. 24.)	20

The dry points of this manufacturer have no date. They were planted December 5, 1902.

The glycerinated virus also had no date and was counted December 2, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 22.—This staphylococcus was one of 853 colonies which grew on our agar plates planted from a dry point of manufacturer No. 8.

The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon cloudy, with white precipitate; on agar, white, shiny, moist, glistening growth; on potato, yellowish white growth; in glucose bouillon, no fermentation, slight growth at bend of tube; in lactose bouillon, no fermentation, slight growth at bend of tube.

Coccus No. 23.—This staphylococcus was one of 887 colonies which grew on our agar plates planted from a dry point of manufacturer No. 8.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; very slight acid production in litmus milk; liquefies gelatine very slowly; bouillon cloudy, with white precipitate; on agar, dirty white, shiny, moist growth; on potato, yellow spreading growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 24.—This staphylococcus was one of 3,754 colonies which grew on our agar plates planted from a dry point of manufacturer No. 8.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; slight acid production in litmus milk; liquefies gelatine; bouillon is cloudy, with white precipitate; on agar there is a white, shiny, moist, glistening growth; on potato, a white granular growth spreading all over the potato; in glucose agar, no fermentation, but growth at bend of tube; in lactose bouillon, no fermentation, but growth at bend of tube.

Coccus No. 25.—This staphylococcus was one of 16 colonies which grew on our agar plates planted from a tube of manufacturer No. 8.

The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; very strong acid production in milk; does not liquefy gelatine; bouillon slightly cloudy, with some white precipitate; on agar, a slight granular scanty growth with no pigmentation; on potato, no growth; in glucose bouillon, no fermentation, growth all through; in lactose bouillon, no fermentation, growth at bend of the tube.

MANUFACTURER NO. 9.

Number of organisms found.	
Dry points.	Glycerinated virus.
5,952	7
7,632	10
7,560	13
7,960	25
8,748 (Coc. 26.)	43 (Coc. 29.)
8,748 (Coc. 27.)	47
9,744	70
11,020	81 (Coc. 30.)
12,972 (Coc. 28.)	85
19,872	105

According to the label, the dry points were prepared by the manufacturer October 31, 1902, and were planted November 29, 1902.

The glycerinated virus was not dated. It was counted December 1, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 26.—This staphylococcus was one of 8,748 colonies which grew on our agar plates, planted from a dry point of manufacturer No. 9.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; is slightly acid in litmus milk; does not liquefy gelatine; bouillon cloudy, with yellow precipitate; on agar, an orange moist glistening growth; on potato, heavy yellow growth; in glucose bouillon, no fermentation, growth all through; in lactose bouillon, no fermentation, growth all through.

Coccus No. 27.—This staphylococcus was one of 8,748 colonies which grew on our agar plates, planted from a dry point of manufacturer No. 9.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; slight acid production in litmus milk; does not liquefy gelatine; in bouillon, heavy white pellicle, with precipitate; on agar, an orange-yellow moist glistening growth; on potato, a yellowish granular growth; no fermentation, cloudy all through, in glucose bouillon; and in lactose bouillon there is no fermentation, but the bouillon is cloudy all through.

Coccus No. 28.—This staphylococcus was one of 12,972 colonies which grew on our agar plates, planted from a dry point of manufacturer No. 9.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk there is a strong acid production, coagulation, and a precipitate of casein; rapidly liquefies gelatine; in bouillon, cloudy with yellow precipitate; on agar, an orange moist glistening growth; on potato, a heavy yellow growth; in glucose bouillon, no fermentation, but cloudy all through; in lactose bouillon, no fermentation, cloudy all through.

Coccus No. 29.—This staphylococcus was one of 43 colonies which grew on our agar plates, planted from a tube of manufacturer No. 9.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk, a strong acid production, coagulation, and precipitate of casein; very rapidly liquefies gelatine; bouillon slightly cloudy with yellow precipitate; on agar, an orange-yellow moist glistening growth; on potato, yellowish slowly spreading growth; in glucose bouillon, no fermentation, but growth all through; in lactose bouillon, no fermentation, but growth all through.

Coccus No. 30.—This staphylococcus was one of 81 colonies which grew on our agar plates, planted from a tube of manufacturer No. 9.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; acid production in litmus milk; liquefies gelatine very slowly; in bouillon, slightly cloudy, with yellow precipitate; on agar, a yellowish brown moist glistening growth; on potato, yellowish slowly spreading growth; in glucose bouillon, no fermentation, growth all through; in lactose bouillon, no fermentation, but growth at bend of tube.

MANUFACTURER NO. 10.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined.	7
	14
	15
	17
	21
	22
	22
	31
	31 (Coc. 31.)
	53

This glycerinated virus was labeled by the manufacturer to be good until January 1, 1903, and was planted December 4, 1902.

The following organisms, resembling the pyogenic cocci, were isolated:

Coccus No. 31.—This staphylococcus was one of 31 colonies which grew on our agar plates planted from a tube of manufacturer No. 10.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk, produces alkali with light yellow precipitate; does not liquefy gelatine; bouillon clear, with some yellow precipitate; on agar, a heavy yellow, shiny growth; on potato, a scanty yellow growth; in glucose bouillon, no fermentation, but slightly cloudy at bend of tube; in lactose bouillon, no fermentation, slightly cloudy at bend of tube.

MANUFACTURER NO. 11.

Number of organisms found.	
Dry points.	Glycerinated virus.
283 (Coc. 32.)	
324	
333 (Coc. 33.)	
684	
728	
3,696	
9,072	
24,360 (Coc. 34.)	
29,145	
44,000	

These points had no date at all. They were planted November 19, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 32.—This staphylococcus was one of 283 colonies which grew on our agar plates, planted from a dry point of manufacturer No. 11.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk there is a slight acid production, coagulation, and a precipitate of casein; rapidly liquefies gelatine; in bouillon, slightly cloudy, with some yellow precipitate; on agar, orange yellow granular growth; on potato, yellow granular spreading growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth all through tube.

Coccus No. 33.—This staphylococcus was one of 333 colonies which grew on our agar plates, planted from a dry point of manufacturer No. 11.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; strong acid production in litmus milk; does not liquefy gelatine; in bouillon, slightly cloudy, with some white precipitate; on agar, a white shiny growth; on potato, nearly invisible white growth; in glucose bouillon, no fermentation, slight growth at bend of tube; in lactose bouillon, slight growth at bend of tube.

Coccus No. 34.—This staphylococcus was one of 24,360 colonies which grew on our agar plates, planted from a dry point of manufacturer No. 11.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk there is a strong acid production, coagulation, and precipitate of casein; slowly liquefies gelatine; bouillon is cloudy, with heavy white precipitate; on agar, a whitish yellow, shiny moist growth; on potato, yellow growth with white border; in glucose bouillon, no fermentation, growth all through tube; in lactose bouillon, no fermentation, growth all through.

SUMMARY OF SERIES III.

November and December, 1902.

Number of organisms found.				
Dry points.		Glycerinated virus.		
0	1,204	0	9	
0	1,399	0	10	
1	1,452	0	10	
1	1,800	1	11	
2	1,847	1	11	
3	1,946	2	11	
3	2,074	2	12	
3	2,244	2	13	
3	2,246	2	13	
3	2,584	2	14	
5	2,912	2	14	
5	3,696	2	15	
5	3,754	2	16	
6	5,952	2	16	
7	7,465	2	16	
11	7,632	3	17	
21	7,560	3	19	
31	7,980	3	19	
48	8,702	3	20	
67	8,748	3	21	
243	8,748	4	22	
262	9,072	4	22	
283	9,744	4	25	
324	10,022	4	31	
333	10,265	4	31	
420	10,982	4	31	
424	11,020	4	37	
452	12,923	4	41	
465	12,972	5	43	
475	15,552	5	47	
479	19,872	5	53	105
526	24,360	5	70	112
576	25,574	5	70	113
604	26,411	6	77	152
684	29,145	6	81	230
728	31,380	6	85	239
810	<u>44,000</u>	6	87	<u>236</u>
853		7	95	
868		7		
887		7		
1,088		7		
1,156		8		
1,199		9		
		9		
Number points examined.. 80		Number tubes examined..... 89		
Total bacteria..... 391,603		Total bacteria..... 2,592		
Average per point..... 4,895		Average per tube..... 29		

DRY POINTS AND GLYCERINATED VIRUS COMPARED FROM A BACTERIOLOGICAL STANDPOINT.

The question is often asked, Is glycerinated virus superior to dry points?

As the object of glycerinating the virus is to eliminate the foreign bacteria, the answer to this question resolves itself almost entirely into a comparative study of the number and kind of organisms found to contaminate this kind of vaccine.

The result of our studies on this point may be summarized as follows:

Series.	Average number of organisms.	
	Dry points.	Glycerinated virus.
Series I, winter, 1901-2	4,809	4,698
Series II, spring, 1902	3,458	1,058
Series III, winter, 1902-3	4,895	29

It is plain that last winter (Series I) the glycerinated virus contained a number of contaminating bacteria equal in amount to the dry points. This winter (Series III) shows a great improvement in the glycerinated virus and demonstrates conclusively that this form of vaccine may be, and should be freer from impurities than the dry points.

Of course the greater freedom from bacterial impurities of the glycerinated virus to the dry points refers only to those dry points made with fresh "lymph." There can be little choice between the dry points made with glycerinated pulp and the capillary tubes containing the same material.

The pyogenic cocci and other bacteria pathogenic for laboratory animals were found both in the glycerinated virus and on the dry points.

EXPERIMENTAL TETANUS IN VACCINE VIRUS.

Can tetanus be found in the vaccine virus bought in open market?

Many tubes containing glycerinated virus and many points of the dry vaccine were examined bacteriologically for tetanus, but we were unable to discover this organism.

This part of the work was done in several ways. Susceptible animals were inoculated and vaccinated with the contents of many tubes

and dry points. Sometimes the total contents of 10 or 12 tubes were injected subcutaneously into guinea pigs or mice. In another series the virus from ivory points or capillary tubes was planted in fresh broth and grown anaërobically, and the resulting growth studied for end-spore-forming rods and also inoculated into animals. In no case was an organism or were symptoms resembling tetanus found.

While these results were most gratifying, they must not be taken as conclusive that tetanus may not sometimes contaminate vaccine virus, although from our studies this must be exceedingly rare. Tetanus organism can not grow or produce its toxin either in the glycerinated virus or on the dry points. It would take gross carelessness to contaminate the vaccine with a sufficient number of tetanus spores to carry the disease to those vaccinated.

It is not a matter of surprise that some outbreaks of tetanus have occurred when thousands of open wounds are presented for the reception of this infection so widely distributed in nature. Local outbreaks or small epidemics of tetanus have been known before the days of antiseptics, due to causes so well understood nowadays, and it seems more likely that carelessness in the dressing or handling of the wound, the pernicious use of shields, or faulty technique in performing the operation has introduced the tetanus spores, than that they were contained in the vaccine virus.

Most of the following work on tetanus was done under my direction by Dr. Edward Francis, and it is a pleasure to acknowledge the care and faithfulness with which he carried out all the exacting details.

VIABILITY OF TETANUS IN VACCINE VIRUS.

Can tetanus live and grow in vaccine virus?

In order to answer this question we contaminated vaccinal serum and the glycerinated pulp with tetanus spores. The serum was dried upon ivory points and the glycerinated pulp was conserved in capillary tubes in a manner similar to the methods now used by the manufacturers. From time to time these points and tubes were examined.

For the purposes of these experiments it was thought best to use a pure culture of tetanus for one series of experiments and for another series to use a mixed culture composed of tetanus, a coccus, and a motile, spore-bearing, saprophytic rod, as representing more nearly the natural conditions under which tetanus would be found to gain entrance into vaccine.

The pure culture of tetanus used in these experiments was taken from our laboratory stock culture. It was planted into ordinary bouillon on March 2 and grown anaërobically in a Novy jar at 37° for seven days, when it contained abundant spores.

VIRULENCE OF THE TETANUS CULTURE USED.

In order to determine the virulence of the pure culture of tetanus used it was tested in white mice, with the following results. Each

quantity of pure bouillon culture indicated was inoculated into the right side of a mouse.

Mouse.	Quantity of tetanus culture injected on March 9.
1.....	c. c. 0.000065
2.....	.000049
3.....	.000039
4.....	.000032
5.....	.000028
6.....	.000024
7.....	.000021

On March 10 the three mice which had received the largest doses showed typical symptoms of tetanus, while the four mice which had received the smallest doses showed no symptoms.

On March 11 the mouse which received the largest dose was dead. The second, third, fourth, fifth, and sixth showed typical symptoms of tetanus, while the seventh, which received the smallest dose, gave no symptoms whatever of tetanus.

On March 12 mice 2 and 3 were dead; 4, 5, 6, and 7 show typical tetanus.

On March 13 mice 4, 5, and 6 were dead; 7 shows typical tetanus.

On March 14 mouse 7 died.

Table showing virulence of the tetanus culture used in these experiments.

Mouse.	March 9.	March 10.	March 11.	March 12.	March 13.	March 14.
1	c. c. 0.000065	Typical tetanus.	Dead.			
2	.000049do.....	Typical tetanus.	Dead.		
3	.000039do.....do.....	Do.		
4	.000032	Negativedo.....	Typical tetanus.	Dead.	
5	.000028do.....do.....do.....	Do.	
6	.000024do.....do.....do.....	Do.	
7	.000021do.....	Negativedo.....	Typical tetanus.	Dead.

From these tests we considered 0.000021 c. c. to constitute about the minimum fatal dose of our culture for an average-sized white mouse.

VIABILITY OF A PURE CULTURE OF TETANUS IN GLYCERINATED VACCINE VIRUS.

The vaccine pulp was taken from the heifer on February 15 and subjected to the action of glycerin by the vaccine manufacturer for twenty-five days. It was then, on March 12, obtained by us and contaminated with our pure culture of tetanus in the following manner:

We aimed to imitate as closely as possible the ordinary conditions under which we find glycerinated vaccine on the market. So we drew out in the flame a large number of capillary tubes of the length and caliber ordinarily used by manufacturers to contain material sufficient for one vaccination. Definitely measured amounts of the pure culture of tetanus were added to large drops of the glycerinated virus, and this mixture was drawn up into the small capillary tubes, which were hermetically sealed in the usual manner. In this way we had an accurate gauge of the exact amount of tetanus put into each capillary tube.

These tubes were filled with the glycerinated virus and tetanus spores on March 12. Each capillary tube contained one drop of glycerinated virus plus the following amounts of tetanus culture:

[.000021=MLD, minimal lethal dose.]

First series	=0.000055 c. c. (about 2½ MLD).
Second series	.00011 c. c. (about 5 MLD).
Third series	.00022 c. c. (about 10 MLD).
Fourth series	.00040 c. c. (about 20 MLD).
Fifth series	.00074 c. c. (about 40 MLD).
Sixth series	.013 e. c. (about 500 MLD).

The capillary tubes were tested immediately in mice as a control and then placed in the cool chamber at 20°–22° C., protected from light, and again tested at intervals in mice and in cultures, as indicated in the following tables:

Capillary tubes containing 0.000055 c. c. of tetanus culture added to glycerinated virus, March 12, and tested at intervals as shown.

[P=positive, i. e., symptoms of tetanus; N=negative, i. e., no symptoms; †=death.]

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.								Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	
Mar. 12	Mar. 12	0	P	P	P	P	P	†	Positive; death.
Do.....	Apr. 11	30	P	P	†	Do.
Do.....	May 21	70	P	P	P	P	†	Do.
Do.....	June 17	97	N	P	P	P	P	P	P	†	Do.
Do.....	July 19	129	N	P	P	Positive; recovered.
Do.....	Aug. 1	142	N	N	P	P	P	Do.
Do.....	Aug. 28	169	N	Negative.
Do.....	Sept. 3	175	N	Do.

We see from this series that the tetanus spores neither multiplied nor produced toxin. In fact, they gradually lost virulence. Each capillary tube in this series of experiments contained approximately two and a half times the minimal lethal dose of the tetanus culture. It

offer a more favorable medium for preserving the vitality of the tetanus spores.

The sterile ivory points were prepared in the usual manner with the vaccinal serum but contaminated with definite measured amounts of tetanus culture.

Each point of the first series contained 0.00012 c. c. of pure bouillon culture of tetanus; each point of the second series contained 0.0002 c. c. of pure bouillon culture of tetanus; each point of the third series contained 0.0004 c. c. of pure bouillon culture of tetanus; each point of the fourth series contained 0.001 c. c. of pure bouillon culture of tetanus.

These were allowed to dry in the usual manner and kept in the cool chamber at 20°–22° C., well protected from the light. These points were tested at intervals on mice and in cultures as shown in the following tables:

Dry points containing vaccine serum ("lymph") were contaminated with 0.00012 c. c. of tetanus culture and kept at 22° C. in the dark. From time to time they were tested, with the following result:

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Mar. 12	May 28	77	N	Negative.
Do.....	June 17	107	N	Do.
Do.....	June 27	117	N	Do.

It will be seen that a quantity of tetanus spores equal to five times the minimal lethal dose lost its toxicity for mice within seventy-seven days. The spores, however, were not dead, for these points planted in bouillon and grown anaerobically in a Novy jar for one week gave a culture rich in end spore-bearing rods, which caused typical tetanus in a mouse in doses of 0.001 c. c.

Dry points containing vaccine serum ("lymph") were contaminated with 0.0002 c. c. of tetanus culture and kept at 22° C. in the dark. From time to time they were tested, with the following result:

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Mar. 12 ..	May 28 .	77	N	Negative.
Do.....	June 17.	107	N	Do.
Do.....	June 27.	117	N	Do.

These points, containing approximately ten times the minimal fatal dose of tetanus, apparently soon lost their virulence when inoculated

Each capillary tube in this series contained about twenty times the minimal fatal dose of tetanus, which lost its virulence after ten months.

Capillary tubes containing 0.00074 c. c. of tetanus culture added to glycerinated virus, March 12, and tested at intervals as shown.

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Mar. 12	Sept. 1	173	P	P	†	Positive; death.
Do...	Oct. 1	203	P	P	P	P	P	P	P	P	P	†	Do.
Do...	Nov. 15	248	P	P	†	Do.
Do...	Jan. 1	295	P	P	†	Do.
Do...	Feb. 1	326	N	P	†	Do.
Do...	Mar. 2	355	N	P	†	Do.

It will be seen that this large amount of tetanus (about forty times the minimal fatal dose) remained alive and virulent in glycerinated virus one year.

Capillary tubes containing 0.013 c. c. of tetanus culture added to glycerinated virus, March 12, and tested at intervals as shown.

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Mar. 12	Sept. 1	173	P	†	Positive; death.
Do...	Oct. 1	203	P	†	Do.
Do...	Nov. 15	248	P	†	Do.
Do...	Jan. 1	295	P	†	Do.
Do...	Feb. 1	326	P	†	Do.
Do...	Mar. 2	355	N	P	†	Do.

This large amount (about $500 \times \text{MLD}$) has also remained alive and virulent for mice one year. The material is not yet exhausted, and will be tested from time to time.

VIABILITY OF PURE CULTURE OF TETANUS ON DRY POINTS.

The serum which exuded from the site of the inoculation after the superficial layers of the variolous eruption was removed was obtained from a calf on March 12, 1902. This serum, commonly called "lymph," when dipped or brushed upon the ivory points and allowed to dry, formerly constituted the "dry points" of the manufacturers. At present some dry points are made with glycerinated virus alone or with glycerinated pulp mixed with blood serum and in other ways. We, however, preferred to use the "lymph" or serum, as it seemed to

for two weeks. The resulting growth was then tested on mice to determine its virulence. Each of two mice were given 0.00002 of the culture. Both animals took the disease. The one died in eight days; the other recovered. One month later, viz, May 28, two more mice were inoculated with an equal amount of the same culture, and both died. This amount was, therefore, considered to represent approximately the minimal fatal dose, and was used to contaminate the vaccine virus.

The glycerinated virus of two manufacturers was bought in the open market. With the mixed tetanus culture we prepared an artificial contamination of both these viruses and sealed them hermetically in small capillary tubes. By means of dilution each capillary tube was made to contain just 0.00002 c. c. of the tetanus culture in addition to a large-sized drop of the glycerinated virus.

These tubes were kept at 20°-22° C. in a dark place and tested from time to time.

Glycerinated virus (manufacturer No. A) contaminated with 0.00002 c. c. of a mixed culture of tetanus.

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Apr. 30	Apr. 30	0	P	P	P	P	P	P	P	P	P	P	Positive; recovered.
Do..	May 28	28	N	Negative.
Do..	June 17	48	N	Do.
Do..	June 27	58	N	Do.

We see from this table that one month's action of the glycerinated virus reduced the virulence of the tetanus which it contained to a point where it no longer produced any symptoms on mice.

A comparison of the table above with the control which immediately precedes it indicates strikingly the attenuating power of the glycerinated virus in this instance. Observe that on May 28 the glycerinated tube produced no symptoms of tetanus, while the two control tests made on the same date both proved typical tetanus. Whether the virus had killed the tetanus or merely diminished its virulence was determined by planting on July 8 six of the tubes each into a separate cubic centimeter of bouillon and growing it under anaërobic conditions for one week. At the end of this time we tested the growth in mice and were surprised to find that three of the tubes when injected into mice caused no sickness whatever, while the other three tubes produced typical symptoms of tetanus in mice.

We conclude that the glycerinated virus killed the tetanus entirely in some of the tubes in two months and that if it had been allowed to act longer on the other tubes they, too, probably would have contained no living tetanus.

Glycerinated virus (manufacturer No. B) contaminated with 0.00002 c. c. of a mixed culture of tetanus.

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Apr. 30..	Apr. 30	0	P	P	P	P	(†)	Positive; death.
Do.....	May 28	28	P	P	P	P	P	P	P	P	P	P	Positive; recovered.
Do.....	June 17	48	N	P	P	P	P	P	P	(†)	Positive; death.
Do.....	June 27	58	N	P	P	P	P	P	P	P	P	(†)	Do.
Do.....	...do....	58	N	N	N	P	P	P	P	P	P	P	Positive; recovered.
Do.....	July 18	79	N	N	P	P	P	P	P	P	P	P	Do.
Do.....	Aug. 1	93	N	N	P	P	P	P	P	P	P	P	Do.
Do.....	Aug. 28	120	N	Negative.
Do.....	Sept. 3	126	N	Do.
Do.....	Sept. 6	129	N	Do.
Do.....	Sept. 29	152	N	Do.

This table shows that after August 28 the tubes failed to produce tetanus in the mice. Therefore, on September 30, we subjected the remaining two tubes to cultivation in bouillon for one week and found that in one of the tubes the tetanus was still alive, for the growth showed rods bearing end spores and produced typical tetanus in mice.

In the other tubes there was no growth of tetanus.

A comparison of these two tables shows that make No. B of glycerinated virus required four months to attenuate the same amount of tetanus which make No. A attenuated in one month and killed completely in two months. This fact led to a study of the comparative effects of the different percentages of pure glycerin on the tetanus organism and its toxin. This study will be reported upon later.

VIABILITY OF TETANUS IN MIXED CULTURE ON DRY POINTS.

The same mixed culture of tetanus used in the two preceding experiments was also used to contaminate dry points. The tips of the sterilized ivory points were dipped into vaccine serum freshly obtained from the calf. To each point was then immediately added a definite measured amount of the tetanus culture. The points were allowed to dry in the air and kept in a cool (20° – 22° C.), dark place until tested.

To contaminate the first series 0.001 c. c. of the tetanus culture was used; 0.0004 c. c. of the tetanus culture was used to contaminate the second series; 0.0002 c. c. of the tetanus culture was used to contaminate the third series; 0.00012 c. c. of the tetanus culture was used to contaminate the fourth series.

Series of dry points containing various amounts of mixed culture of tetanus, prepared June 2.

Amount of tetanus culture.	Inoculated into mice.	Result.
c. c.		
0.001	June 17	Remained negative.
	June 27	Do.
.0004	June 17	Do.
	June 27	Do.
.0002	June 17	Do.
	June 27	Do.
.00012	June 17	Do.
	June 27	Do.

Compare this table with the control experiments on page 28 and we see the striking attenuating effect of exposure of tetanus on dry points.

For instance: On May 28 two tests of the control culture killed mice with typical symptoms of tetanus; whereas, on June 17, after an exposure of two weeks on dry points, doses of tetanus which represented as much as fifty times the control doses had been rendered nontoxic for mice. It now remained to determine whether the tetanus on the point was dead or only attenuated. It was found after growing a point of each series in 1 c. c. of bouillon for one week that the growth showed end-spore bearing organisms in microscopic preparations and produced typical symptoms of tetanus when inoculated into mice.

Again, on October 4, four months after the dry points were prepared, specimens from series 0.0004, 0.0002, and 0.00012 c. c. were planted into bouillon and grown anaërobically. The culture again contained end spore bearing organisms which, inoculated into mice, produced typical symptoms of tetanus. The tetanus spores on the points were therefore not dead, although they had lost their virulence for mice when inoculated directly.

SUMMARY AND CONCLUSIONS.

We examined the vaccines of 10 different manufacturers during a period of more than a year.

All the samples examined were purchased on the open market, care being taken to buy unbroken original packages from reliable pharmacists who keep the product under proper conditions of light and temperature. With a few exceptions, the vaccine was examined before the time limit expired, if the time was given by the manufacturer.

Of 190 dry points examined we found an average of 4,354 bacteria per point. A number of these points contained over 15,000 and one as high as 44,000 organisms.

Of 244 tubes of glycerinated virus examined we found an average of 1,742 bacteria per tube. A number of these capillary tubes contained over 10,000 bacteria and one as high as 30,000. This is of course much more than a carefully prepared glycerinated virus should contain.

We found the pus cocci and other bacteria, pathogenic for laboratory animals, in both the dry points and the glycerinated virus.

We have demonstrated that some of the glycerinated virus marketed during the winter of 1901-2 contained an excessive number of bacteria, which decreased notably after a few weeks, indicating the sale of a "green" or unripe product; that is to say, the virus was not glycerinated a sufficient length of time before it was sold.

It was also plain from our studies that too much confidence was placed by the producers in the germicidal power of glycerin.

The excessive impurities found in some of the glycerinated virus upon the market we believe was largely due to this overconfidence in the germicidal value of glycerin; operators become careless, trusting to the glycerin to purify their product. We know that glycerin is too feeble in its properties to purify vaccine matter which has initial contamination such as our work indicates.

After we called attention to this point we found a marked improvement in the glycerinated virus on the market. For instance:

During the winter of 1901-2 the glycerinated virus contained an average of 4,698 bacteria per tube.

In the spring (April and May) of 1902 the average fell to 1,058 bacteria per tube.

This winter (November and December), 1902, the average of 89 tubes examined was only 29 bacteria—maximum 239.

Glycerinated virus, when properly prepared and kept a sufficient length of time, is freer from impurities than dry points made with fresh "lymph."

There is practically no difference between the glycerinated virus dried upon ivory points and that hermetically sealed in capillary tubes, so far as bacteriological impurities are concerned.

It will be seen that there is practically no vaccine on the market free from bacterial contamination, although the product of some manufacturers is remarkably pure.

The unevenness of the purity of vaccine as marketed by some manufacturers is very marked. The greater contamination of the vaccine during the winter of 1901-2 may be accounted for, in part, by the undue haste and accompanying carelessness incidental to supplying the increased demand for vaccine virus at that period.

We have examined a great number of dry points and the contents of glycerinated tubes for tetanus, but have been unable to find the organism of this disease.

Tetanus spores may live a long time in vaccine virus. We have found them alive and virulent on dry points after two hundred and ninety-five days and in glycerinated virus sealed in capillary tubes three hundred and fifty-five days.

Tetanus may become a contaminating element of vaccine before it leaves the heifer. During the period of three to five days which elapses between the vaccination of the heifer and the removal of the virus there is opportunity for tetanus to find a lodgment in the eruption on the heifer's body surface, provided tetanus is present in the stall or stable surroundings of the animal.

If tetanus reaches the heifer's vaccinated area it may contaminate both the "dry points," which are made directly from the "lymph," and also the vaccine pulp, which the manufacturer subjects for a longer or shorter time to the germicidal action of glycerin before he markets it as "glycerinated virus."

Tetanus added to glycerinated vaccine virus does not germinate when kept hermetically sealed and under anaërobic conditions in small capillary tubes. It gradually loses its virulence both in the tubes and on the ivory points. Although the virulence is lost, the tetanus spores are not necessarily dead; for, while they will not produce symptoms when inoculated directly into mice, they may be revived into active virulent cultures by growing in fresh bouillon under favorable conditions. That is to say, the vegetability of the spore remains active long after it has lost its power to produce the disease when inoculated directly into mice.

Therefore, in looking for tetanus in vaccine virus it is best to make cultures first and study the growth for end spore-bearing rods and then test the effects of the culture in animals.

The spores of tetanus lose their virulence and die much more quickly in the vaccine lymph on dry points than in the glycerinated tubes.

The vitality of tetanus in glycerinated virus depends largely upon the number of spores contaminating the virus. Large quantities, namely, forty times the minimal lethal dose ($40 \times \text{MLD}$), remain alive and active over a year. Smaller amounts may disappear in four to seven months. Very small amounts ($1 \times \text{MLD}$) have in one of our experiments lost virulence in one month and failed to grow in bouillon in two months.

On the other hand, these very small amounts of tetanus may remain active for months in glycerinated virus in capillary tubes. In one instance as small a quantity as 0.000055 c. c., which was about two and a half times the minimal fatal dose, remained active seven months.

In vaccinal "lymph" on dry points the spores may begin to lose their virulence in two weeks and be dead in two months. Usually they live longer. We found them alive and virulent in points that had been contaminated with $50 \times \text{MLD}$ about ten months (two hundred and ninety-five days).

TREASURY DEPARTMENT.

Public Health and Marine-Hospital Service of the United States.

WALTER WYMAN, Surgeon-General.

HYGIENIC LABORATORY.—BULLETIN No. 13.

M. J. ROSENAU, Director.

May, 1903.

A STATISTICAL STUDY OF THE INTESTINAL PARASITES
OF 500 WHITE MALE PATIENTS AT THE UNITED
STATES GOVERNMENT HOSPITAL FOR THE INSANE.

By PHILIP E. GARRISON, BRAYTON H. RANSOM,
AND EARLE C. STEVENSON.

A PARASITIC ROUNDWORM (*Agamomermis culicis* n. g., n. sp.)
IN AMERICAN MOSQUITOES (*Culex sollicitans*).

By CH. WARDELL STILES.

THE TYPE SPECIES OF THE CESTODE GENUS *Hymenolepis*.

By CH. WARDELL STILES.



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1903.

ORGANIZATION OF HYGIENIC LABORATORY.

WALTER WYMAN, *Surgeon-General,*
United States Public Health and Marine-Hospital Service.

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A STATISTICAL STUDY OF THE INTESTINAL PARASITES OF 500 WHITE MALE PATIENTS AT THE UNITED STATES GOVERNMENT HOSPITAL FOR THE INSANE.

By PHILIP E. GARRISON, A. B., BRAYTON H. RANSOM, M. A., and EARLE C.
STEVENSON, B. Sc.,
Assistants in the Division of Zoology, Hygienic Laboratory, U. S. Public Health and
Marine-Hospital Service.

SUMMARY OF RESULTS.—(1) The results show 13.2 per cent of the patients examined infected with intestinal parasites. The parasites found were hookworms (*Uncinaria americana* or *Agchylostoma duodenale*^a), whipworms (*Trichuris trichiura*), seatworms (*Oxyuris vermicularis*), Cochinchina worms (*Strongyloides stercoralis*), and eelworms (*Ascaris lumbricoides*). No evidence of infection with tapeworms, flukes, or coccidia was found. (2) Our results differ from those of most foreign investigators principally in the lower rate of infection, in the absence of tapeworms, and in the presence of hookworms and of the Cochinchina worms. (3) The results show that the percentage of infection tends to vary inversely with the age and with the duration of institutional life of the patients. (4) They also indicate that army life is conducive to parasitic infection of the intestine, and, moreover, that a high percentage of the United States soldiers returning from service in the Philippine Islands have intestinal parasites. (5) The presence of a moderate number of worms in the intestine seems to have no relation to the presence of undigested starch and meat in the dejecta or to the litmus reaction of the feces.

INTRODUCTION.—By the courtesy of Dr. A. B. Richardson, superintendent of the Government Hospital for the Insane, a microscopic examination of the feces of the patients in that institution was begun early in

^a It could not be ascertained with certainty in some cases, from measurement of the ova, that the hookworms belonged to the species *Uncinaria americana*, as the number of the eggs was small and their size somewhat varying. In the great majority of infections, however, the eggs were found to be full 64 by 40 μ or larger, and we would have little hesitation in calling them *Uncinaria americana* rather than *Agchylostoma duodenale* in every case, were it not for the fact that most of the patients had been in the Philippines, where the Old World form (*Agchylostoma duodenale*) has been found. Final judgment as to the species of the hookworms must be reserved, therefore, until the adult worms can be obtained and examined.

September, 1902, by the Division of Zoology of the Hygienic Laboratory under the direction of Dr. Ch. Wardell Stiles, chief of the Division. The examinations were for the purpose of determining the percentage of intestinal infections with animal parasites and of demonstrating the value of microscopic examination of the feces in general clinical diagnosis. The following results have been obtained from 500 white male patients, and include the number and kinds of infection found with regard to age, length of residence of the patients within the institution, and character of life previous to admission, together with an investigation of the effects of parasitic infection of the intestine upon digestion and upon the litmus reaction of the feces.

FREQUENCY OF INFECTION.—Of the 500 patients examined, 66 patients, or 13.2 per cent, showed parasitic infection of the intestines. Ten patients had a double infection, and in one case three different parasites were present, making a total of 78 infections. These were distributed among five parasites, as follows:

PARASITES PRESENT.—

Uncinaria americana or *Agchylostoma duodenale*^a (hookworms), 15 cases, or 3 per cent of the cases examined.

Trichuris trichiura (whipworms), 54 cases, or 10.8 per cent.

Oxyuris vermicularis (seatworms), 4 cases, or 0.8 per cent.

Strongyloides stercoralis (Cochin-China worms), 3 cases, or 0.6 per cent.

Ascaris lumbricoides (eelworms), 2 cases, or 0.4 per cent.

No evidence of parasitic infection of the liver, stomach, lungs, or other organs was present. No tapeworms, flukes, or coccidia were found.

SEVERITY OF INFECTIONS.—In general it may be said that the cases of infection with intestinal parasites found by us at the Government Hospital were not severe. In one or two cases of hookworm disease several eggs were present under a single cover-glass, but in the majority of cases we found only 5 or 6 eggs under the 10 covers examined, and in no case were the ova so numerous as in the severe infections with this parasite found by Dr. Stiles in the Southern States.^b In some cases of infection with *Trichuris* the eggs were very numerous in the feces and indicated the presence of a great number of worms in the intestine, but as a rule not more than 5 or 6 eggs were found in examining 10 preparations. In one of the infections with *Strongyloides* the feces were rather heavily infested with the embryos. The number of eggs found in the infections with *Oxyuris* and *Ascaris* was small.

^aSee footnote page 5.

^bReport upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States, by Ch. Wardell Stiles, Ph. D., Bull. 10, Hyg. Lab., U. S. Pub. Health & Mar.-Hosp. Serv., Wash., pp. 1-121, figs. 1-86.

The thorough and repeated treatment with purgatives which the patients at the Government Hospital receive may explain the comparatively small number of worms which seem to be present in the infected cases, for in all probability many of the worms are eliminated by repeated purgation.

COMPARISON WITH FOREIGN STATISTICS.—We have at present no American statistics of parasitic infection in adults with which to compare these general results. Following (Table 1) are the tabulated results of similar investigations in Europe and Asia, together with those obtained from the work at the Government Hospital.

TABLE 1.—Statistics of intestinal worms, in United States, Europe, and India.

Authority and locality.	Number of subjects examined.	Hookworms.		Trichuris.		Oxyuris.	
		Num-ber.	Per cent.	Num-ber.	Per cent.	Num-ber.	Per cent.
United States Government Hospital, 1902	500	15	3	54	10.8	4	0.8
Heisig, Germany, 1893	230			104	45.2		
Cima, Italy, 1893, 1896	73			28	38.35	3	4.1
Dobson, India, 1893 ^a	1,249	944	75.58	55	4.41	192	15.37
Kesler, St. Petersburg, 1888	600			30	5	43	7.16
Sievers, Germany, 1887	2,629			521	19.8	326	12.4
Roth, Germany, 1877-1880 ^b	752			178	23.67		
Gribohm, Germany, 1872-1877 ^b	972			313	32.20	226	23.24
Heller, Kiel, 1872-1875	611			187	30.6	142	23.2
Müller, Germany, 1862-1873 ^b	1,755			159	9.05	213	12.13
Müller, Germany, 1852-1862 ^b	1,989			50	2.57	43	2.21
Zaeleln, Germany ^c					23.7		20
Banik, Germany ^c					8.3		30.6

	Strongyloides.		Ascaris lumbricoides.		Tænia.		Total. ^d	
	Num-ber.	Per cent.	Num-ber.	Per cent.	Num-ber.	Per cent.	Num-ber.	Per cent.
United States Government Hospital, 1902	3	0.6	2	0.4			78	15.6
Heisig, Germany, 1893			34	14.7	2	0.87	140	60.77
Cima, Italy, 1893, 1896			22	30.13	5	6.84	58	79.42
Dobson, India, 1893 ^a			131	10.49	18	1.44	1,840	107.29
Kesler, St. Petersburg, 1888			35	5.83		14.51		
Sievers, Germany, 1887			436	16.56	8	.30	1,291	49.06
Roth, Germany, 1877-1880 ^b			86	11.43			264	35.1
Gribohm, Germany, 1872-1877 ^b			178	18.30			717	73.76
Heller, Kiel, 1872-1875			108	17.73			437	71.17
Müller, Germany, 1862-1873 ^b			227	12.93			599	34.11
Müller, Germany, 1852-1862 ^b			180	9.28			273	14.07
Zaeleln, Germany ^c				11.4				
Banik, Germany ^c				7.3				

^aDobson found also 13 cases of distomatosis (1.04 per cent).

^bHeisig, 1893, Beitrag zur Statistik menschlicher Entozoen, Greifswald.

^cSievers, 1887, Schmarotzer-Statistik aus den Sections-Befunden des pathologischen Instituts zu Kiel.

^dIt will be noted that the figures in the total column represent the number of infections found per 100 cases, not the number of cases infected.

In every case but one the foreign results show a higher percentage of infection with all parasites than was found in our examinations. In no case was mention made of *Strongyloides*, and the only statistics for hookworms are those of Dobson in India, who found there 75.58 per cent of infection with *Agchylostoma duodenale* in examining over 1,200 natives.

AGE.—The average age of the men in whom parasites were found was 37.75 years; of the uninfected cases, 47.9 years. The average age of those infected with hookworms was 32.8 years; with *Trichuris*, 36.1 years; with *Oxyuris*, 66 years; with *Strongyloides*, 45.66 years; with *Ascaris*, 41.5 years.

Dividing the patients into classes according to age, we obtain the following results:

TABLE 2.—Frequency of infection with regard to age of patients.

Age (years).	Number examined.	Hookworms.		<i>Trichuris</i> .		<i>Oxyuris</i> .		<i>Strongyloides</i> .		<i>Ascaris lumbricoides</i> .		Total. ^a	
		No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
18-30.....	93	7	7.53	17	18.28	0	0	0	0	0	0	24	25.8
31-50.....	184	4	2.17	26	14.13	0	0	2	1.09	2	1.09	34	18.42
51+.....	194	1	0.52	5	2.53	4	2.01	1	0.52	0	0	11	5.67
Unknown.....	29	3	10.34	6	20.69	0	0	0	0	0	0	9	31.03
Total.....	500	15	3.00	54	10.8	4	0.8	3	0.6	2	0.4	78	15.6

^a See footnote d, table 1.

The rate of infection decreases as the age of the patients increases. Heisig found similar results in Germany, though his percentages were higher and his patients included both men and women.

TABLE 3.

	15 (18)-30 years.			31-50 years.			51+ years.		
	Number examined.	Infected.		Number examined.	Infected.		Number examined.	Infected.	
		No.	Per cent.		No.	Per cent.		No.	Per cent.
Heisig.....	17	7	41.5	28	9	32.1	23	3	13.
Government hospital.	93	24	25.8	184	33	17.9	194	11	5.67

LENGTH OF RESIDENCE IN THE HOSPITAL.—Considering the amount of infection found with regard to the length of residence of the patients within the hospital, the results are as follows: Admitted within one year, 162 men; infected, 28 men, or 17.28 per cent. With from one to three years' residence, 154 men; infected, 20, or 12.99 per cent. From three to eight years, 57 men; infected, 5, or 8.77 per cent. From eight to fifteen years, 45 men; infected, 3, or 6.66 per cent. More than fifteen years, 71 men; infected, 7, or 9.86 per cent.

The rate of infection decreases as the length of residence in the hospital increases, except in the class which had spent fifteen years or more in the institution, where the percentage rises.

The following table gives the frequency of infection with each parasite in each of the above classes:

TABLE 4.—Frequency of infection with regard to length of residence in the hospital.

Number of years.	Number examined.	Hookworms.		Trichuris.		Oxyuris.		Strongyloides.		Ascaris lumbricoides.		Total. ^a	
		No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
-1.....	162	9	5.5	26	16.09	0	0	0	0	1	0.62	36	22.22
1-3.....	154	4	2.67	18	11.69	0	0	3	1.95	0	0	25	16.23
4-8.....	57	1	1.75	5	8.77	0	0	0	0	1	1.75	7	12.28
9-15.....	45	1	2.22	1	2.22	1	2.22	0	0	0	0	3	6.66
15+.....	71	0	0	4	5.63	3	4.23	0	0	0	0	7	9.86
No record.....	11	0	0	0	0	0	0	0	0	0	0	0	0
Total.....	500	15	3.00	54	10.8	4	0.8	3	0.6	2	0.4	78	15.6

^a See footnote d, table 1.

CHARACTER OF LIFE PREVIOUS TO ADMISSION.—Considering the patients examined with regard to the character and conditions of their life immediately previous to admission to the hospital, they fall into four main groups—those admitted from the Army, those admitted from the United States Soldiers' Homes, those admitted from the District of Columbia, and those admitted from the Navy. A fifth or miscellaneous class contains a small number of United States convicts, patients admitted from the United States Territories, and a few men concerning whose previous life no data were obtainable. The Army group is naturally subdivided into three classes: First, men admitted before the outbreak of the Spanish-American war (1898), composed of soldiers from the regular army posts; second, soldiers engaged in that war who served within the States; and, a third and important class, soldiers who had returned from service in the Philippine Islands.^a

Soldiers admitted before 1898.—This class included 30 men, 4 of whom, or 10 per cent, had intestinal parasites. Three infections were with *Trichuris trichiura* and 1 with *Oxyuris vermicularis*.

Soldiers admitted after 1898.—This class also contained 40 men, 9 of whom, or 22.5 per cent, were infected with parasites. Eight infections were with *Trichuris trichiura* and 1 with hookworms.

Soldiers returned from the Philippines.—Fifty-nine men were examined who had returned from service in the Philippines. Twenty-five of these, or 42.46 per cent, had intestinal parasites. Fifteen men were infected with *Trichuris* alone, 1 with hookworms alone, 7 with hookworms and *Trichuris*, 1 with hookworms and *Strongyloides*, and 1 with hookworms, *Trichuris*, and *Ascaris*. Thus in the 59 soldiers

^a Of the soldiers admitted to the Government hospital after service in Cuba and Porto Rico nearly all had been discharged before these investigations were begun, so that the interesting statistics which might be expected from this class were not obtainable.

there were 10 infections with hookworms, 23 infections with *Trichuris*, 1 infection with *Strongyloides*, and 1 infection with *Ascaris*, or a total of 35 infections.

Men admitted from the United States Soldiers' Homes.—Of the 124 men admitted from the United States Soldiers' Homes 8 men, or 6.45 per cent, were infected. Hookworms, *Oxyuris*, *Strongyloides*, and *Ascaris* were each found once, while *Trichuris* was present four times.

Men admitted from the District of Columbia.—This class included 137 men, 11 of whom, or 8.03 per cent, were infected, namely, 9 with *Trichuris*, 1 with *Oxyuris*, and 1 with *Strongyloides*.

Men admitted from the Navy.—Forty-two patients were admitted from the Navy or from naval hospitals. Of this number 4 men, or 9.5 per cent, had intestinal parasites. Hookworms were found once and *Trichuris* was found three times.

Miscellaneous.—Of the 58 patients not classified, 7 men, or 12.07 per cent, showed infection, namely, 2 with hookworms, 4 with *Trichuris*, and 1 with *Oxyuris*.

These results are summarized in the following table:

TABLE 5.—Frequency of infection with regard to character of life previous to admission to the hospital.

Previous history.	No. examined.	Hookworms.		Trichuris.		Oxyuris.		Strongyloides.		Ascaris lumbricoides.		Total. ^a	
		No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
Army before 1898.....	40	0	0	3	7.5	1	2.5	0	0	0	0	4	10.0
Army after 1898.....	40	1	2.5	8	20.0	0	0	0	0	0	0	9	22.5
Philippine Islands.....	59	10	16.95	23	38.98	0	0	1	1.7	1	1.7	35	59.32
Soldiers' Homes.....	124	1	0.81	4	3.23	1	0.81	1	0.81	1	0.81	8	6.45
District of Columbia.....	137	0	0	9	6.57	1	0.73	1	0.73	0	0	11	8.03
Navy.....	42	1	2.38	3	7.14	0	0	0	0	0	0	4	9.52
Others.....	58	2	3.47	4	6.9	1	1.72	0	0	0	0	7	12.09
Total.....	500	15	3.00	54	10.8	4	0.8	3	0.6	2	0.4	78	15.6

^a See footnote ^d, Table 1.

THE SIGNIFICANCE OF AGE, INSTITUTIONAL LIFE, AND THE CONDITIONS OF PREVIOUS LIFE WITH REGARD TO THE AMOUNT OF INFECTION WITH INTESTINAL PARASITES.—The soldiers admitted after service in the Philippine Islands, among whom was found a percentage of infection more than twice as high as in any other class, were for the most part young men, their average age being 30.5 years. They were also patients of recent admission, none having been in the hospital longer than three years. We may discover the relative significance of Philippine service as a condition favorable to infection with intestinal parasites by comparing the Philippine men with that class of patients who during the late war served in the reserve camps situated principally in the Southern States. The average age of this latter class of men was 34.8 years, and all but four had been admitted to the hospital

within three years, thus presenting conditions about the same as found among the Philippine men. The frequency of infection, however, was only 20 per cent, as compared with 42.46 per cent among the soldiers returned from the Philippine Islands. The much higher rate of infection in the Philippine men can be accounted for by the greater prevalence of the parasites in the Philippines and by the poor sanitary conditions under which it was necessary for the men to live during active service in the islands.

The results obtained in these examinations at the Government hospital would indicate that a large number of the United States soldiers returning from service in the Philippine Islands return with a parasitic infection of the intestines. While the percentage of infection with hookworms (16.95 per cent) among these soldiers was not so high as that with whipworms (39.98 per cent), its clinical importance is much greater, because of the severe anemic and nervous symptoms which the hookworm is capable of producing; and the danger of this parasite being spread in this country, especially the danger of its being carried to parts of the country where it does not now exist, makes the presence of hookworm infection among the soldiers returning from the Philippines a matter of moment from the view-point of public health.^a

The patients admitted from the Army after the outbreak of the Spanish-American war show a much higher rate of infection (20 per cent) than do those admitted from the Regular Army before 1898 (10 per cent). In the former class we find an average age of 34.8 years and only four men who had been in the hospital longer than three years. In the latter class the average age was 45.6 years, and all except six of the men had been in the hospital longer than eight years. Accordingly, the lower percentage of infection among the soldiers admitted before the war seems to be due to a combination of three factors, namely, greater age, longer period of institutional life, and the better sanitary and hygienic conditions found at the Regular Army posts than in the temporary reserve camps established during the war.

Considering the 124 patients who had been admitted to the Government hospital from the United States Soldiers' Homes, we find the highest average age (62.9 years) of any of the classes studied, and while 97 of the 124 men had been received at the hospital within three years prior to our examination, all of them had resided for a longer or shorter period at the Soldiers' Homes, so that we have a combina-

^aSince this report was prepared the feces of 9 soldiers newly arrived at the Government hospital from the Philippines have been examined for parasites. Six of the 9 men were infected, 1 with hookworms alone, 1 with hookworms and *Trichuris*, 2 with *Trichuris* alone, 1 with *Ascaris lumbricoides* alone, and 1 with *Ascaris lumbricoides* and *Trichuris*.

tion of old age and a long period of institutional life. In this class we found the lowest rate of infection (6.45 per cent).

The 137 civilians admitted to the hospital from the District of Columbia were rather evenly distributed both with regard to age and to length of residence in the institution. Their average age was 42.5 years. Seventy-four men had been admitted within four years, and 59 had had longer than four years' residence in the hospital. Of the 137 men 8.03 per cent were found infected. The 74 patients who had been admitted within four years showed 9.46 per cent of infection, while the 59 patients with a longer residence showed only 6.78 per cent.

These results are lower than those obtained by Sommer in the District of Columbia in 1895. (Southern Journal of Homœopathy, December, 1895, pp. 353-354.) In examining 36 children, ranging from 1 month to 14 years in age, in the Children's Hospital of the District, he found 11.11 per cent to have intestinal parasites. All the infections were with *Trichuris*. The difference in age and in condition between his patients and those examined at the Government hospital may well explain the disparity in results.

In the 42 patients admitted from the United States Navy we find again a younger class of men, averaging 34.6 years, and men of comparatively recent admission to the hospital, 25 of the 42 having been received within three years prior to our examination. Although the average age and the average length of institutional life are lower in this class than in the soldiers admitted from the Regular Army, the percentage of infection is lower, for which we must hold accountable the conditions of their life prior to admission.

DIGESTION.—In the investigation of the relation of parasitic infection of the intestines to the presence of undigested food in the feces our results were entirely negative. In 28 cases large quantities of undigested starch granules were found in the stools and in 7 cases a large quantity of undigested meat fibers. Of these 35 cases only 6 were infected with parasites. On the other hand there were 61 men with parasites present in the intestine who did not show this evidence of poor digestion of starch or meat.

LITMUS REACTION OF FECES.—The litmus test was made of the feces of 324 patients. Of the 270 uninfected cases tested 79.26 per cent were alkaline, 15.93 per cent acid, and 4.81 per cent gave no reaction. Of 54 cases tested where parasites were present 81.48 per cent were alkaline, 12.77 per cent acid, and 5.55 per cent gave no reaction. About the same results were obtained by considering the infections with each parasite separately, alkaline and acid reaction being present in practically the same proportions as in the uninfected feces. The results, therefore, must be considered as entirely negative.

TECHNIQUE.—Some difficulty was anticipated in obtaining the fecal specimens and in conveying them from the hospital into the city to

the laboratory, where the examinations were to be made, but as the work progressed the following methods were developed and very little trouble was experienced:

The attendants on the wards were provided with aluminum chambers into which the stools were passed in the morning before or immediately after breakfast, when the patients generally desire to defecate. The stubbornness and the suspicious ideas of the patients in many cases were difficult to overcome, but there were very few cases where by patience and persistence on the part of the attendants the specimen could not be obtained. The chambers containing the feces were set aside in a vacant room by the attendants.

From one to two grams was taken from each specimen (preferably from the surface) and wrapped in a piece of common wrapping paper. These papers being thoroughly wrapped together in a large piece of paper made a small package which could be carried handily and without odor. In case the excrement were fluid, small glass jars with screw tops were used. To transfer the specimen to the paper or jar we made use of 1 by 3 inch glass slides, which were cleanly and could be readily washed and reused, or of stiff cardboard slips.

Ten preparations were made from each specimen for examination with the microscope, using the following technique: The large 2 by 3 inch glass slides were used, these being cleaner and more easily handled than the ordinary 1 by 3 inch slide. A drop of distilled water was placed on each slide, and with this a portion of the feces little larger than a pin head was thoroughly mixed by rubbing with the end of a glass rod. On this preparation was placed a three-quarter inch cover glass (square preferred) of medium thickness. Two preparations were made on each slide and then examined with the microscope while fresh without staining, using a lens of moderate power, such as the Zeiss 8 mm. or the Zeiss C. To insure greater accuracy at least two men examined preparations from each specimen. The examination of ten specimens (100 preparations) is an average day's work for one person.

A PARASITIC ROUNDWORM (AGAMOMERMIS CULICIS N. G., N. SP.) IN AMERICAN MOSQUITOES (CULEX SOLLICITANS).

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At a time when mosquitoes are subjected to such careful study, because of the important relations they bear to public health, especially in connection with malaria, yellow fever, etc., it is of interest to determine what parasites naturally infest them. This determination has its practical as well as its scientific value, for it enables us to eliminate certain nonpathogenic parasitic organisms from the life cycle of pathogenic organisms, stages of which may be found in mosquitoes. It further has its direct practical bearing in that the parasites of mosquitoes may multiply to such an extent as to become important factors in killing the insects, or at least in rendering them less fertile.

Quite recently several parasites have been described for the Culicidæ. Ross (1895) has found intestinal gregarines in mosquito larvæ in India. Perroncito (1899) has found a filamentous phytoparasite in *Anopheles* collected near Turin, Italy. Laveran (1902) has described a pathogenic yeast in the abdominal cavity of *Anopheles maculipennis* collected in Spain, and he reports various acarines as external parasites of the Culicidæ. Léger (1902) has described a parasitic flagellate (*Crithidia fasciculata*) in the intestine of the adult female of *Anopheles maculipennis*. Herbert Johnson (1902) has described a sporozoon as infecting about 8 per cent of the females of *Anopheles maculipennis* collected in a certain locality in Massachusetts in which tertian malaria is endemic. Martirano (1901) has described a minute trematode (*Agamodistomum Martiranoi*^a Stiles, 1903 [new name]) found in the body cavity of *Anopheles claviger* (= *A. maculipennis*) taken in Italy. G. W. Mueller found an undetermined sporozoon of the genus *Glugea* in *Culex*.

To these cases of parasitism I am now able to add another of considerable interest. Prof. John B. Smith, of Rutgers College, has

^aMartirano, 1901, Centralbl. f. Bakteriöl., Parasitenk. [etc.], Jena, v. 30 (23), 24. Dec., pp. 849-852, figs. 1-4.

kindly forwarded to me for examination two worms taken from the abdominal cavity of *Culex sollicitans*. One specimen was hardly in condition to be of use in study, but it represented a larval roundworm, probably either a *Mermis* or a *Paramermis*. The second specimen was determined as a larval roundworm, but owing to the fact that its genital organs were not developed, its exact generic position could not be recognized. It apparently belongs in the family Mermithidæ, either to *Mermis* or to *Paramermis*. It will be convenient to recognize for these larval forms a special biological group, for which I propose the name *Agamomermis*.

The characters of the groups in question may be summarized as follows:

Family MERMITHIDÆ.

FAMILY DIAGNOSIS.—Thread-like worms, quite similar to *Filaria* in general appearance. Mouth with 6 papillæ. In adults, the posterior end of the intestinal canal is more or less atrophied. Male with 1 or 2 spicules and with numerous caudal papillæ arranged in three or four rows. Larvæ parasitic, especially in the abdominal cavity of arthropods; adults free living.

TYPE GENUS.—*Mermis* Dujardin, 1842.

Genus MERMIS Dujardin, 1842.

GENERIC DIAGNOSIS.—Mermithidæ: Male with two equal spicules.

TYPE SPECIES.—*Mermis nigrescens* Dujardin, 1842.

Genus PARAMERMIS von Linstow, 1898.

GENERIC DIAGNOSIS.—Mermithidæ: Male with one spicule.

TYPE SPECIES.—Not determined—either *P. crassa* (von Linstow, 1889) or *P. aquatilis* (Dujardin, 1845).

Group AGAMOMERMIS Stiles, 1903.

GENERIC DIAGNOSIS.—Mermithidæ: An artificial collective group containing larval forms which can not be more definitely determined because of lack of genital organs. As such a group is artificial it should have no type species.

Species AGAMOMERMIS CULICIS Stiles, 1903.

SPECIFIC DIAGNOSIS.—*Agamomermis*: About 11 mm. long; 240 μ in diameter. Caudal spine 88 μ long.

HABITAT.—Abdominal cavity of mosquitoes (*Culex sollicitans*), New Jersey.

TYPE SPECIMEN.—Collection U. S. P. H. & M.-H. S., No. 9401,^a in poor condition; collected by Dr. John B. Smith, New Brunswick, N. J.

^a **IMPORTANT NOTICE TO HELMINTHOLOGISTS.**—In order to prevent confusion, and following the precedent of the United States Bureau of Animal Industry, the specimens of parasites and of other objects in medical zoology which become property of the Division of Zoology, Hygienic Laboratory, United States Public Health and Marine-Hospital Service, will be given numbers in the series of the Helminthological Collection of the United States National Museum.

Nos. 1 to 4700 have been set aside for the United States Bureau of Animal Industry.

Nos. 4701 to 9400 have been set aside for the miscellaneous specimens of parasites deposited in or presented to the United States National Museum or sent to the Museum for determination.

Nos. 9401 to — have been set aside for the United States Public Health and Marine-Hospital Service.

In the summer of 1889 I collected a number of specimens of *Agamomermis* sp. from mosquitoes of the species *Culex nemoralis* taken in the vicinity of Leipzig, Saxony. Whether they were identical with the present form I am unable to state. The interesting fact may be mentioned, however, that the Leipzig *Agamomermis* was decidedly injurious to the mosquitoes. It was found in the abdominal cavity of larvæ, pupæ, and adults, so that infection must have taken place in the water, namely, in the larval and pupal stages of the *Culex*. The infested insects were very sluggish in their movements and could usually be easily recognized as diseased. Many of them died from the effects of the parasite, and the ovaries of infected females were underdeveloped. Professor Leuckart informed me at that time that he had frequently found *Culex nemoralis* infected with this worm, and that during the years that the worms were most common the mosquitoes seemed to be less numerous.

These cases represent interesting instances in nature, where a pest is subject to other pests which tend to hold the former in check.

- At least two other species of *Mermis* should be placed in the collective group *Agamomermis*, namely, *Agamomermis gammari* (von Linstow, 1892), parasitic in *Gammarus pulex*, and *A. sialidis* (von Linstow, 1892), parasitic in *Sialis lutaria*.

Bibliography.—Bibliographic citations follow the references in Stiles & Hassall, Index-Catalogue of Medical and Veterinary Zoology, Bull. 39, Bureau Animal Industry, U. S. Dept. Agric., Wash.

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THE TYPE SPECIES OF THE CESTODE GENUS HYMENOLEPIS.

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The determination of type species for certain genera is not unattended with difficulties and with differences of opinion. *Hymenolepis*, for example, presents an instance in regard to which helminthologists are not in accord. The premises of the case are these:

(1) Weinland (1858, § 68, pp. 49 to 57) proposed the genus *Hymenolepis*. (2) He did not specifically state that any particular species was chosen as a type; (3) but he combined only one specific name, namely, *flavopunctata* (= *Tænia diminuta*), with *Hymenolepis* (see Weinland, 1858, pp. x, 16, 49, 52, 53, 55, 57, 58, 75, 85, 92). (4) His discussion of the genus is based on this form. (5) In a footnote, pp. 50 to 53, to this discussion, he reviews the classification of certain tapeworms and, p. 52, he mentions *Hymenolepis*. (6) This genus he there divides into two subgenera, namely, (7) Subgenus 1. *Lepidotrias*, stating "As the type, we may consider *Tænia murina* Dujardin; and besides this belong here *Tænia scalaris*, ----, and *Hymenolepis flavopunctata*." (8) Subgenus 2. *Dilepis* -----, "and we may consider *Tænia angulata* Rudolphi as its type." (9) In *Dilepis* he also placed, besides other species, "the Tænioid of the golden-winged woodpecker, mentioned above, § 28." (10) The latter form is evidently *Liga punctata*, described in 1857 as new species from *Picus auratus* (=the flicker, *Colaptes auratus* (Linnæus, 1758), Vigors, 1827), as is seen from the description of the testicles. (11) *Liga punctata* is type and only species of *Liga* Weinland, 1857.

In this case, therefore, at the date of 1858 we have four generic names (*Hymenolepis*, 1858; *Lepidotrias*, 1858; *Dilepis*, 1858; and *Liga*, 1857) to deal with, and of these Weinland should undoubtedly have used *Liga* instead of proposing *Hymenolepis*.

Types were definitely fixed for *Lepidotrias*, namely, *Tænia murina* Dujardin, 1845 (not Gmelin, 1789) = *Tænia nana* von Siebold, 1852, which is also type of *Diplacanthus* Weinland, 1858 (not Agassiz, 1842); for *Dilepis*, namely, *T. angulata*; and for *Liga*, namely, *L. punctata*.

As the subgenus *Dilepis*, 1858, contained the older genus *Liga*, 1857, Weinland should not have proposed *Dilepis*; but since he definitely

proposed *T. angulata* as type, the case comes under § 7 of the Stricklandian code, which states: "Provided, however, that if these authors select their respective types from different sections of the genus, and these sections be afterward raised into separate genera, then these names may be retained in a restricted sense for the new genera, respectively." Thus as long as *Liga* and *Dilepis* are considered generically or subgenerically identical, *Liga*, 1857, takes precedence over *Dilepis*, 1858; but if *Liga* and *Dilepis* are recognized as generically distinct, both names are available (but not necessarily valid) for the respective genera or subgenera.

In determining the type of *Hymenolepis* we have before us a case of practically the same nature. Weinland has definitely designated *Tænia murina* as type of *Lepidotrias*, but he has placed *Hymenolepis flavopunctata* in this subgenus. The genus *Hymenolepis* itself is based directly upon *flavopunctata*, as is clearly shown by the reference, "Gen. 1. *Hymenolepis* Weinland (see § 68)," for § 68 is the discussion of *H. flavopunctata*, and this reference "(see § 68)" can, and I believe it should, be interpreted as designation of the type. Thus, if *Hymenolepis flavopunctata* (= *T. diminuta*) and *Tænia murina* (= *T. nana*) are congeneric, *Lepidotrias* is synonym of *Hymenolepis*; but should these two species ever be recognized as generically distinct, both names are available in determining the valid names of the respective genera.

In 1896 I published *flavopunctata*=*diminuta* as type of *Hymenolepis*, basing my action on the above interpretation, but not publishing the details.

At that time the fact had escaped my attention that Blanchard (1891a) had mentioned *T. murina*=*T. nana* as type. Blanchard's reasoning was apparently based on the view that *Hymenolepis* had been divided into two subgenera, both of the latter having type species; hence either *murina* or *angulata* should be type of *Hymenolepis*.

There is a certain amount of justice in this point of view, and it must be admitted that one of Weinland's (1861, pp. 1-24) papers lends considerable support to it. Still this interpretation does not appear to correspond altogether with Weinland's earlier publications or intentions. In chronological sequence Weinland's text (§ 68) was surely written before his footnote. The fact that he consistently combined *flavopunctata*, but no other species, with *Hymenolepis* is significant. Further, if *flavopunctata* is taken as type of *Hymenolepis*, the name *Lepidotrias* is not hopelessly suppressed, while if *murina*=*nana* is taken as type of *Hymenolepis* we needlessly forfeit all use of *Lepidotrias* unless *Hymenolepis* should eventually prove to be a homonym.

In order to obtain the views of other zoologists who have made a special study of the principles involved in determining type species, I have laid this case, with the original literature in question, before Drs. Merriam and Palmer (mammalogists and ornithologists), Dr. Stejneger

(ornithologist and herpetologist), Dr. Gill (ichthyologist), Dr. Dall, (conchologist), and Dr. Benedict and Miss Rathbun (both of whom have given especial attention to the types of crustacea). Of these 7 specialists, none of whom knew which position Blanchard supported and which one I adopted, Dr. Palmer inclined to the view that *murina* (= *nana*) should be the type of *Hymenolepis*; Dr. Benedict thought that it might be either *murina* or *flavopunctata*, and all the other 5 thought that no question could be raised since it seemed so evident that Weinland himself looked upon *flavopunctata* as type of *Hymenolepis*.

Braun (1900a, pp. 1669, 1717) accepts *Tænia diminuta* as type of *Hymenolepis*, thus adopting the ruling I made in 1896.

Bibliography.—Bibliographic citations follow the references in Stiles & Hassall, Index-Catalogue of Medical and Veterinary Zoology, Bull. 39, Bureau Animal Industry, U. S. Dept. Agric., Wash.

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